

BEST SYNTHETIC METHODS

# Carbohydrates

H. M. I. OSBORN



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# **Carbohydrates**

# BEST SYNTHETIC METHODS

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# Carbohydrates

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*For Tariq, Ali and Sara*

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# Contents

<b>Chapter 1</b>	<b>An Introduction to Carbohydrate Synthesis, <i>Helen Osborn</i> . . . . .</b>	<b>1</b>
	1.1 Biological roles of carbohydrates . . . . .	1
	1.2 Synthesis of carbohydrates . . . . .	5
	References . . . . .	7
<b>Chapter 2</b>	<b>Selective Hydroxyl Protection and Deprotection, <i>Jeremy Robertson and Petra M. Stafford</i> . . . . .</b>	<b>9</b>
	2.1 Introduction . . . . .	9
	2.2 Hydroxyl group reactivity . . . . .	10
	2.3 Ethers . . . . .	11
	2.4 Esters . . . . .	22
	2.5 Carbonates . . . . .	28
	2.6 Acetals . . . . .	29
	2.7 Experimental procedures . . . . .	33
	References . . . . .	65
<b>Chapter 3</b>	<b>Synthesis and Activation of Carbohydrate Donors: Acetates, Halides, Phenyl selenides and Glycals, <i>Benjamin G. Davis, David Chambers, Ian Cumpstey, Robert France and David Gamblin</i> . . . . .</b>	<b>69</b>
	3.1 Introduction . . . . .	69
	3.2 Acetates . . . . .	69
	3.3 Halides . . . . .	76
	3.4 Phenyl selenides . . . . .	95
	3.5 Glycals . . . . .	109
	References . . . . .	118
<b>Chapter 4</b>	<b>Synthesis and Activation of Carbohydrate Donors: Thioglycosides and Sulfoxides, <i>K.P. Ravindranathan Kartha and Robert A. Field</i> . .</b>	<b>121</b>
	4.1 Introduction . . . . .	121
	4.2 Thioglycosides . . . . .	121
	4.3 Sulfoxides . . . . .	137
	References . . . . .	142
<b>Chapter 5</b>	<b>Synthesis and Activation of Carbohydrate Donors: Acetimides, <i>n</i>-Pentenyl and Vinyl Glycosides, <i>Antony J. Fairbanks and Christopher M.P. Seward</i> . . . . .</b>	<b>147</b>
	5.1 The trichloroacetimidate method . . . . .	148
	5.2 Use of <i>n</i> -pentenyl glycosides as glycosyl donors . . . . .	165
	5.3 Use of vinyl glycosides as glycosyl donors . . . . .	181
	5.4 Overall summary . . . . .	191
	References . . . . .	192

<b>Chapter 6</b>	<b>Modern Glycosidation Methods: Tuning of Reactivity,</b>	
	<i>Jörg Pietruszka</i> .....	195
	6.1 Introduction .....	195
	6.2 Influence of protecting groups .....	197
	6.3 Influence of leaving group .....	205
	6.4 Summary .....	211
	References .....	216
<b>Chapter 7</b>	<b>Modern Glycosidation Methods: Orthogonal Glycosidation,</b>	
	<i>Jörg Pietruszka</i> .....	219
	7.1 Introduction .....	219
	7.2 Orthogonal glycosidations .....	220
	References .....	237
<b>Chapter 8</b>	<b>The Stereoselective Synthesis of <math>\beta</math>-Mannosides, Seth C. Ennis</b>	
	<i>and Helen M.I. Osborn</i> .....	239
	8.1 The natural occurrence of $\beta$ -mannosides .....	239
	8.2 Problems associated with $\beta$ -mannoside synthesis .....	241
	8.3 Synthetic methods .....	242
	8.4 Conclusion .....	274
	References .....	274
<b>Chapter 9</b>	<b>Synthesis of Sialic Acid Containing Carbohydrates, Hiromune Ando,</b>	
	<i>Hideharu Ishida and Makoto Kiso</i> .....	277
	9.1 Introduction .....	277
	9.2 Sialic acid donors .....	278
	9.3 Sialylation in oligosaccharide synthesis .....	289
	References .....	305
<b>Chapter 10</b>	<b>The Synthesis of Glycosyl Amino Acids, Philip G. Evans,</b>	
	<i>Natasha Gemmell and Helen M.I. Osborn</i> .....	311
	10.1 Introduction .....	311
	10.2 <i>O</i> -Glycosylamino acids and <i>O</i> -glycopeptides .....	311
	10.3 <i>N</i> -Glycosylamino acids and <i>N</i> -glycopeptides .....	317
	10.4 Solid phase synthesis of <i>N</i> - and <i>O</i> -glycosylamino acids and <i>N</i> - and <i>O</i> -glycopeptides .....	322
	10.5 Conclusion .....	323
	10.6 Experimental procedures .....	323
	References .....	333
<b>Chapter 11</b>	<b>The Synthesis of <i>C</i>-linked Glycosides, Paul Meo</b>	
	<i>and Helen M.I. Osborn</i> .....	337
	11.1 Introduction .....	337
	11.2 Synthetic methods .....	339
	11.3 Conclusion .....	381
	References .....	381

**Chapter 12    The Uses of Glycoprocessing Enzymes in Synthesis,**  
                  *Benjamin G. Davis and Susan M. Hancock* ..... 385

          12.1    Introduction ..... 385

          12.2    Glycosidases ..... 387

          12.3    Glycosyltransferases ..... 413

          12.4    Overall summary ..... 423

          References ..... 423

**Index** ..... 427

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# Detailed Contents

<b>1 An Introduction to Carbohydrate Synthesis</b>	<b>1</b>
1.1 Biological roles of carbohydrates	1
1.2 Synthesis of carbohydrates	5
References	7
 <b>2 Selective Hydroxyl Protection and Deprotection</b>	 <b>9</b>
2.1 Introduction	9
2.2 Hydroxyl group reactivity	10
2.3 Ethers	11
2.3.1 Benzyl ethers	13
2.3.2 Allyl ethers	17
2.3.3 Silyl ethers	20
2.4 Esters	22
2.4.1 Acetates	23
2.4.2 Substituted acetates	25
2.4.3 Benzoates	27
2.5 Carbonates	28
2.6 Acetals	29
2.7 Experimental procedures	33
References	65
 <b>3 Synthesis and Activation of Carbohydrate Donors: Acetates, Halides, Phenyl selenides and Glycals</b>	 <b>69</b>
3.1 Introduction	69
3.2 Acetates	69
3.2.1 Preparation of acetates	70
3.2.2 Use of anomeric acetates as glycosyl donors	74
3.3 Halides	76
3.3.1 Preparation of glycosyl bromides and chlorides	77
3.3.2 Use of glycosyl bromides and chlorides as glycosyl donors	82
3.3.3 Preparation of glycosyl fluorides	84
3.3.4 Use of glycosyl fluorides as glycosyl donors	91
3.4 Phenyl selenides	95
3.4.1 Preparation of phenyl selenides	96
3.4.2 Glycosidation chemistry of phenyl selenoglycosides	102
3.5 Glycals	109
3.5.1 Preparation of glycals	109
3.5.2 Use of glycals as direct glycosyl donors: haloglycosylation	114
3.5.3 Using glycals to form epoxide glycosyl donors: 1,2-anhydrosugar glycosylation	116
References	118



<b>4 Synthesis and Activation of Carbohydrate Donors: Thioglycosides and Sulfoxides</b>	121
4.1 Introduction	121
4.2 Thioglycosides	121
4.2.1 Preparation	121
4.2.2 Procedures	123
4.2.3 Activation	128
4.3 Sulfoxides	137
4.3.1 Preparation and activation	137
4.3.2 Procedures	138
References	142
<b>5 Synthesis and Activation of Carbohydrate Donors: Acetimides, <i>n</i>-Pentenyl and Vinyl Glycosides</b>	147
5.1 The trichloroacetimidate method	148
5.1.1 Preparation of trichloroacetimidates	149
5.1.2 Glycosylation chemistry of trichloroacetimidate donors	155
5.1.3 The stereochemical outcome of the glycosylation reaction	156
5.1.4 Glycosylation reactions with trichloroacetimidates: summary	164
5.2 Use of <i>n</i> -pentenyl glycosides as glycosyl donors	165
5.2.1 Preparation of <i>n</i> -pentenyl glycosides	165
5.2.2 Glycosylation chemistry of <i>n</i> -pentenyl glycosyl donors	170
5.2.3 Reactivity of <i>n</i> -pentenyl glycosides in oligosaccharide synthesis i: latent and active <i>n</i> -pentenyl glycosides	172
5.2.4 Reactivity of <i>n</i> -pentenyl glycosides in oligosaccharide synthesis ii: armed and disarmed <i>n</i> -pentenyl glycosides	179
5.2.5 Glycosylation reactions with <i>n</i> -pentenyl glycosides: summary	181
5.3 Use of vinyl glycosides as glycosyl donors	181
5.3.1 Isopropenyl glycosides as glycosyl donors	182
5.3.2 A latent/active glycosylation strategy using vinyl glycosides	184
5.3.3 Glycosylation reactions with vinyl glycosides: summary	191
5.4 Overall summary	191
References	192
<b>6 Modern Glycosidation Methods: Tuning of Reactivity</b>	195
6.1 Introduction	195
6.2 Influence of protecting groups	197
6.2.1 Electronic effects	197
6.2.2 Torsional effects	199
6.2.3 Experimental details	202
6.3 Influence of leaving group	205
6.3.1 Steric effects	206
6.3.2 Electronic effects	206
6.3.3 Experimental details	208
6.4 Summary	211
6.4.1 Application in oligosaccharide synthesis	211
6.4.2 Experimental details	214
References	216

<b>7 Modern Glycosidation Methods: Orthogonal Glycosidation</b>	219
7.1 Introduction	219
7.2 Orthogonal glycosidations	220
7.2.1 Use of glycosyl bromides	221
7.2.2 Use of glycosyl fluorides	222
7.2.3 Primary activation by Lewis acids	223
7.2.4 Summary	224
7.2.5 Experimental details	227
References	237
 <b>8 The Stereoselective Synthesis of <math>\beta</math>-Mannosides</b>	 239
8.1 The natural occurrence of $\beta$ -mannosides	239
8.2 Problems associated with $\beta$ -mannoside synthesis	241
8.3 Synthetic methods	242
8.3.1 The use of insoluble promoters	242
8.3.2 The use of 2-oxo glycosyl halides	246
8.3.3 $S_N2$ Displacement of $\alpha$ -mannosyl triflates	251
8.3.4 Dibutylstannylene complexes	254
8.3.5 The use of glycosyl phosphate donors	259
8.3.6 Intramolecular aglycon delivery	261
8.3.7 Reductive cleavage of cyclic orthoesters	271
8.4 Conclusion	274
References	274
 <b>9 Synthesis of Sialic Acid Containing Carbohydrates</b>	 277
9.1 Introduction	277
9.2 Sialic acid donors	278
9.2.1 Standard type donors	278
9.2.2 Appended type donors	286
9.3 Sialylation in oligosaccharide synthesis	289
9.3.1 Sialylation with an oligosaccharide acceptor	289
9.3.2 Sialyl building blocks	289
9.3.3 Experimental details	292
References	305
 <b>10 The Synthesis of Glycosyl Amino Acids</b>	 311
10.1 Introduction	311
10.2 <i>O</i> -Glycosylamino acids and <i>O</i> -glycopeptides	311
10.2.1 Chemical synthesis of <i>O</i> -glycoamino acids and <i>O</i> -glycopeptides	313
10.3 <i>N</i> -Glycosylamino acids and <i>N</i> -glycopeptides	317
10.3.1 Chemical synthesis of <i>N</i> -glycoamino acids and <i>N</i> -glycopeptides	318
10.3.2 Formation of the <i>N</i> -glycosidic bond	318
10.4 Solid phase synthesis of <i>N</i> - and <i>O</i> -glycosylamino acids and <i>N</i> - and <i>O</i> -glycopeptides	322
10.5 Conclusion	323
10.6 Experimental procedures	323
References	333

<b>11 The Synthesis of C-linked Glycosides</b>	337
11.1 Introduction	337
11.2 Synthetic methods	339
11.2.1 Use of the anomeric centre as a nucleophilic species	340
11.2.2 Reductive samariation method	351
11.2.3 Use of the anomeric centre as an electrophilic species	355
11.2.4 1,2-Anhydro sugars as electrophilic donors	367
11.2.5 Intermolecular radical reactions	371
11.2.6 Intramolecular radical reactions	374
11.3 Conclusion	381
References	381
<b>12 The Uses of Glycoprocessing Enzymes in Synthesis</b>	385
12.1 Introduction	385
12.2 Glycosidases	387
12.2.1 Preparation of activated donors	389
12.2.2 Experimental procedure for preparation of activated donors	390
12.2.3 Temperature and use of organic solvent	392
12.2.4 Solvent volume	392
12.2.5 Product trapping	392
12.2.6 Optimisation of time	393
12.2.7 Regioselectivities	393
12.2.8 Purification	394
12.2.9 Recycling the enzyme	394
12.2.10 General notes on the practical use of glycosidases	394
12.2.11 Examples of thermodynamically controlled reverse hydrolysis reactions	395
12.2.12 Examples of kinetically controlled transglycosylation reactions	400
12.2.13 The use of glycosynthases	410
12.3 Glycosyltransferases	413
12.3.1 Donor synthesis	415
12.3.2 Gly-T catalysed glycosylation	416
12.4 Overall summary	423
References	423
<b>Index</b>	427

## Abbreviations

Ac	acetyl
All	allyl
Alloc	allyloxycarbonyl
aq.	aqueous
Ar	unspecified Aryl substituent
Asn	asparagine
ATP	adenosine 5'-triphosphate disodium salt
BDA	butane diacetal
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
BSA	bovine serum albumin
Bu	butyl
<sup>t</sup> Bu	<i>tert</i> -butyl
Bz	benzoyl
c	concentration
C.I.	chemical ionisation
CAN	ceric ammonium nitrate
cat.	catalytic
CDA	cyclohexane-1,2-diacetal
CDP	cytidine diphosphate
ClAc	chloroacetyl
CMP	cytidine 5'-monophosphate
conc.	concentrated
COSY	correlation spectroscopy
CSA	(+/-)-10-camphorsulfonic acid
d	doublet
δ	chemical shift
DAST	diethylamino sulfurtrifluoride
DBU	1,5-diazabicyclo[4.3.0]non-5-ene
DCC	dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHP	dihydropyran
dispoke	dispiroketal
DMAP	4-dimethylamino pyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide

DMPM	3,4-dimethoxybenzyl
DMSO	dimethylsulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
DTBMP	2,6-di- <i>t</i> -butyl-4-methylpyridine
Dts	dithiasuccinoyl
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
equiv.	stoichiometric equivalents
Et	ethyl
FAB	fast atom bombardment
Fmoc	9-fluorenylmethyloxycarbonyl
Fuc	fucose
Gal	galactose
GalNAc	galactosamine
GDP	guanidine diphosphate
Glc	glucose
GlcNAc	glucosamine
h	hours
Hz	Hertz
IDCP	iodonium di- <i>sym</i> -collidine perchlorate
<sup>i</sup> Pr	<i>iso</i> -propyl
IR	infra red
<i>J</i>	coupling constant
KDN	3-deoxy-D- <i>glycero</i> -D- <i>galacto</i> -non-2-ulosonic acid
LDMAN	lithium 1-(dimethylamino) naphthalenide
Lev	levulinate
lit.	literature value
LN	lithium naphthalenide
m	multiplet
m	<i>meta</i>
M	molar concentration
MCPBA	<i>meta</i> -chloroperbenzoic acid
m.p.	melting point
m/z	mass to charge ratio
Me	methyl
Man	mannose
min	minutes
mol	mole
MS	molecular sieves
Ms	mesyl
NBA	<i>N</i> -bromoacetamide
NBB	<i>N</i> -bromobenzamide
NBP	<i>N</i> -bromophthalimide
NBS	<i>N</i> -bromosuccinimide
Neu	neuraminic acid

Neu5Ac	<i>N</i> -acetyl derivative of neuraminic acid
Neu5Gc	<i>N</i> -glycolyl derivative of neuraminic acid
NIS	<i>N</i> -iodosuccinimide
NMR	nuclear magnetic resonance
NPG	<i>n</i> -pentenyl glycoside
NTP	nucleotide triphosphate
<i>o</i>	<i>ortho</i>
<i>p</i>	<i>para</i>
Pfp	pentafluorophenyl
Ph	phenyl
phth	phthaloyl
Piv	pivaloyl
PMB	<i>p</i> -Methoxybenzyl
PNP	<i>para</i> -nitrophenyl
ppm	parts per million
PPTS	pyridinium <i>para</i> -toluenesulfonate
Py	pyridine
R <sub>f</sub>	retention factor
Rha	rhamnose
rt	room temperature
Ser	serine
sLe <sup>x</sup>	sialyl Lewis <sup>x</sup>
S.M.	starting material
sTn	sialyl Tn
t	triplet
TBAF	tetrabutylammonium fluoride
TBAI	tetra butyl ammonium iodide
TBDMS	<i>tert</i> -butyldimethylsilyl
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBPA	tris(4-bromophenyl)ammoniumyl hexachloroantimonate
Teoc	2,2,2-trichloroethoxycarbonyl
TES	triethylsilyl
Tf	triflate
TFA	trifluoroacetic acid
TFAc	<i>N</i> -trifluoroacetyl
TfOH	triflic acid
THF	tetrahydrofuran
THP	tetrahydropyran
Thr	threonine
TIPS	tri- <i>iso</i> -propylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TPS	triphenylsilyl

Tr	trityl
Troc	2,2,2-trichloroethoxycarbonyl
Ts	<i>para</i> -toluene sulfonyl
U	units
UDP	uridine triphosphate
$v_{\max}$	frequency maximum
Xyl	xylose

# An Introduction to Carbohydrate Synthesis

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## 1.1 BIOLOGICAL ROLES OF CARBOHYDRATES

In the past, carbohydrates were considered to be solely of use for energy storage, and as skeletal components. However, this naïve view was challenged in 1963 when a protein was isolated from *Canavalia ensiformis* that demonstrated ability to bind to carbohydrates on erythrocytes. In 1982 the first animal carbohydrate binding protein was identified, and this sparked interest into the wider roles of carbohydrates and carbohydrate binding proteins within biological systems. These carbohydrate binding proteins are termed lectins and it is now known that they are found in varying densities on all cell-surface membranes [1]. They interact specifically with oligosaccharides and glycoconjugates (such as glycolipids and glycoproteins) on the surrounding cells via hydrogen bonding, metal coordination, van der Waals forces, and hydrophobic interactions. It is believed that favourable interactions occur between the hydroxyl groups of the carbohydrates and the amino acid functionalities of the proteins, to aid molecular recognition processes. These interactions are relatively weak, but they are so numerous that specific interactions occur. Selectivity is believed to be further increased through additional binding of the carbohydrate to the lectin's subsites.

The study of carbohydrates within biological systems has illustrated that they are involved in a number of fundamental biological functions such as cell–cell recognition and cell-external agent interactions [2]. These interactions can initiate beneficial biological events [2], such as fertilization, cell growth and differentiation (for example during embryogenesis) [3] and immune responses, as well as detrimental disease processes [2], such as inflammation, viral and bacterial



Therefore the view that carbohydrates are of limited importance within biological systems has been challenged and renewed interest into the science of 'Glycobiology' has emerged.

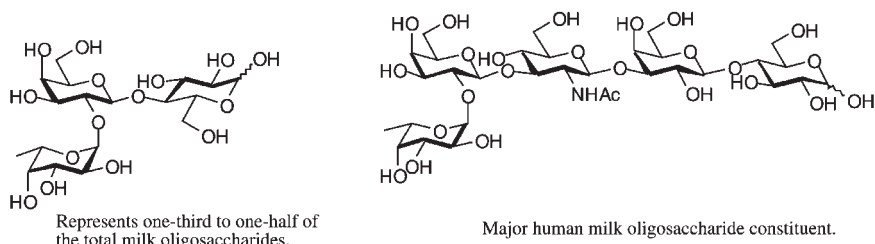
(1) Some bacterial surface proteins demonstrate specific binding for carbohydrates expressed on human cells, and such interactions form an essential part of the infection pathway. It has been demonstrated that administration of synthetic or natural carbohydrate derivatives can disrupt this infective pathway, so long as the administered derivatives have a high affinity for the bacterial lectins [4]. In such cases, the bacteria are no longer able to interact with the host, and therefore pass through the body without initiating infection. Such therapeutic agents have been termed anti-infective agents. A number of anti-infective agents occur naturally, for example, human breast milk contains numerous soluble oligosaccharides that provide newborn babies with a mechanism for aborting infection processes (Figure 1.2) [5].

An alternative approach for treating bacterial infections has seen the development of carbohydrate based antibiotics to target carbohydrate receptors and carbohydrate modifying enzymes [6].

(2) The synthesis of disease associated carbohydrates may hold the key for the development of vaccination strategies for the respective diseases [7]. For example, the use of tumour associated carbohydrate antigens for raising antibodies for the treatment of cancer is currently being developed (Figure 1.3) [8].

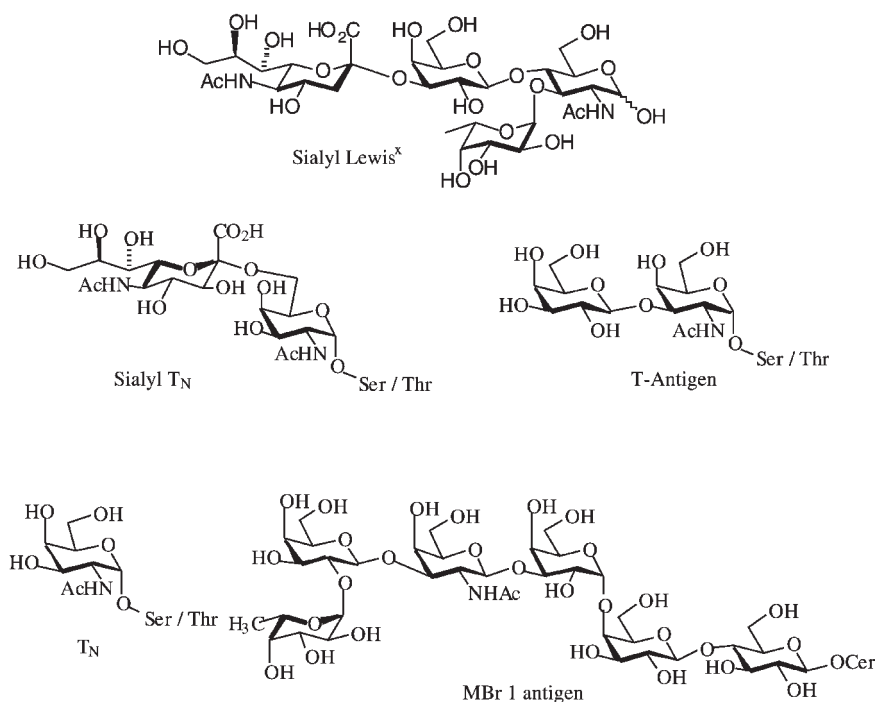
(3) The synthesis of carbohydrate analogues is also being pursued in an attempt to inhibit the interactions between the carbohydrates and selectins that are essential for



**Figure 1.2**

disease progression [9]. This has received particular attention for inhibiting tumour growth and metastasis. The natural role of the selectins is to assist the ‘rolling’ of leukocytes on the surface of activated endothelial cells in the blood vessels. However, the unusual carbohydrates on cancer cells also provide tumour cells with a mechanism for moving along the endothelial cells, in a process known as metastasis [10]. Therefore novel cancer treatments are investigating the possibility of utilizing soluble carbohydrates to block the selectin sites on the epithelial cells in the blood vessels to inhibit metastasis.

An alternative approach has concentrated on aborting the synthesis of disease associated carbohydrates, and this again requires access to carbohydrate

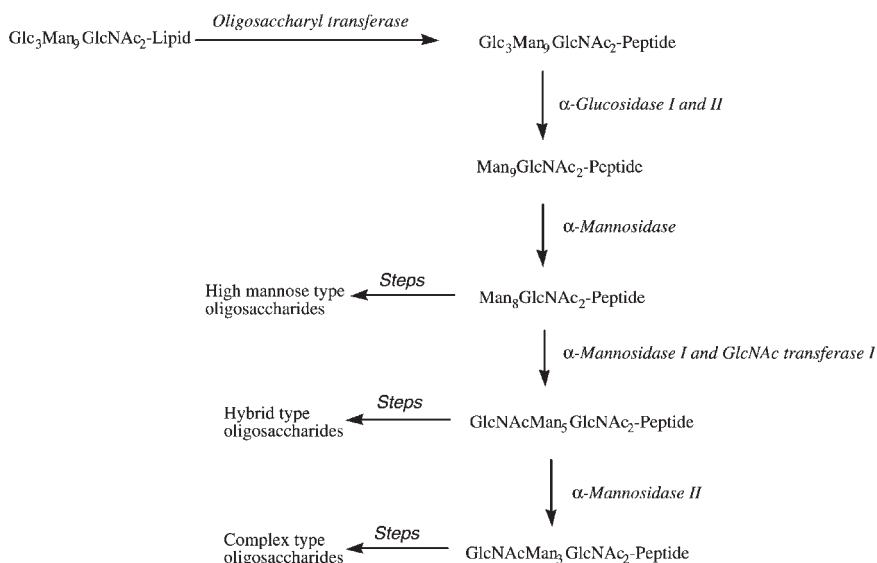
**Figure 1.3**

analogues [11]. The assembly of carbohydrates within biological systems occurs in the endoplasmic reticulum of the golgi apparatus and involves a number of enzyme mediated steps [12]. Biosynthesis commences with the formation of a  $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$  oligosaccharide which is covalently bound to a lipid. This is transferred from the lipid to a peptide via a further enzyme and a series of glycosyl hydrolase and glycosyl transferase enzymes then process the oligosaccharide further to afford a wide range of structurally diverse oligosaccharides (Scheme 1.1).

It has been demonstrated that carbohydrate analogues are capable of inhibiting specific enzymes involved in the carbohydrate biosynthesis, offering the potential to engineer the synthesis of different carbohydrates. This has proved of use for aborting the synthesis of disease associated carbohydrates that are central to the infection pathway [13].

(4) Carbohydrate derivatives have also proved of use for targeting drugs or genes to hepatocytes [14]. An asialoglycoprotein receptor is found on mammalian hepatocytes that has high specificity for ligands displaying terminal Gal or GalNAc residues. Thus liver specific carriers, such as liposomes and polymers that display these residues have been created for the selective delivery of drugs and genes to hepatocytes. In theory this approach could be extended to allow more general carbohydrate-directed therapies [15].

In order to fully explore the biological roles of carbohydrates and exploit the therapeutic opportunities divulged above, it is essential to develop efficient regio- and stereoselective methods for the synthesis of carbohydrates. Whilst carbohydrates can sometimes be isolated from natural sources, synthetic strategies



**Scheme 1.1**

often offer the advantage of allowing access to larger quantities of material as well as entry to analogues of the natural carbohydrates.

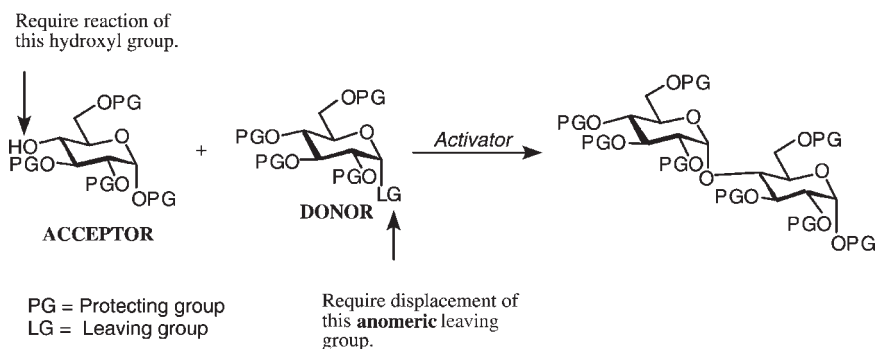
## 1.2 SYNTHESIS OF CARBOHYDRATES

Factors governing the synthesis of saccharides can be appreciated by considering the synthesis of a disaccharide. This generally involves the reaction of a glycosyl acceptor with a glycosyl donor, with an activator being utilized to activate the donor for glycoside bond formation (Scheme 1.2).

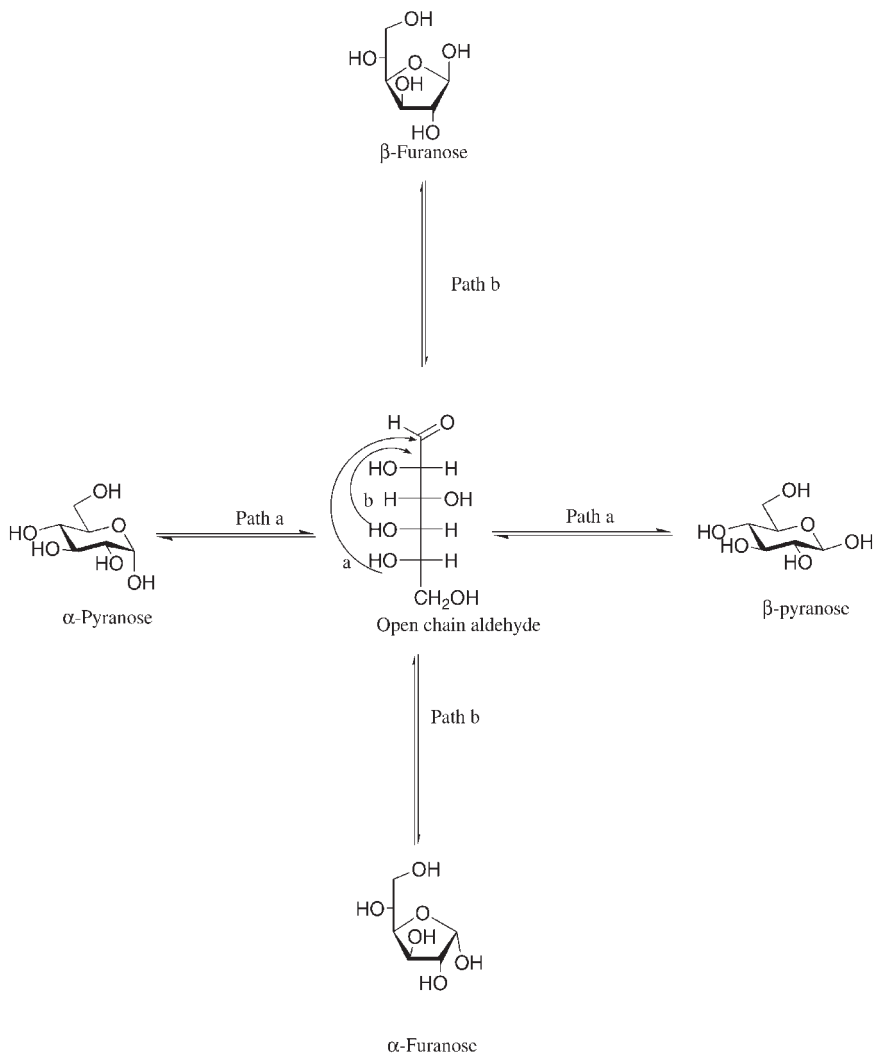
There are a number of factors that make carbohydrates particularly difficult to synthesise and analyse:

1. Carbohydrate building blocks are capable of existing in more than one form (pyranose and furanose forms), and so it is important to control which form is available for reaction (Scheme 1.3).
2. Carbohydrates characteristically have a number of hydroxyl groups which are often of similar reactivity. If no protecting groups are incorporated within the building blocks then mixtures of products will result from chemical synthetic strategies. Hence protecting group strategies are often required to ensure that only the hydroxyl group required for reaction is left in a reactive form. It is essential that the protecting groups are introduced and removed with excellent yield, whilst leaving other parts of the molecule in-tact. This important aspect of carbohydrate synthesis is discussed in Chapter 2.
3. The creation of a new bond between two carbohydrate units offers the potential to form two epimeric (anomeric) isomers (Figure 1.4).

In order to access only the required stereoisomer, it is essential that the geometries of the glycosidic bonds are controlled. Some linkages are particularly difficult to form, for example  $\beta$ -mannosidic linkages, even though they are abundant within



Scheme 1.2

**Scheme 1.3**

natural systems. This specific problem, and some solutions for efficient assembly of the  $\beta$ -mannosidic linkage, are presented in Chapter 8.

There are, however, advantages associated with the synthesis of carbohydrates: a large number of building blocks (both monosaccharide and higher oligomers) are commercially available in large quantities, at economic prices, for incorporation within synthetic strategies. D-Monosaccharides are usually incorporated within natural oligosaccharides rather than the enantiomeric L-series. Also, there are a number of chemical methods that can be utilized to influence

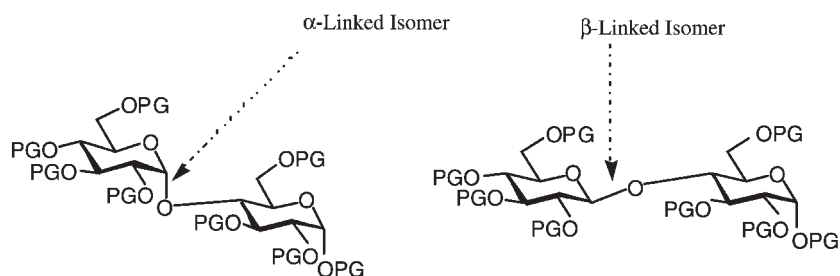


Figure 1.4

which regio- and stereoisomer will result. Although methods for the solid phase synthesis of carbohydrate targets have developed at a slower rate than for peptide and oligonucleotide targets, significant progress has been made recently and it is likely that exciting results will be presented in this area in the near future [16].

Each chapter in this book will detail a particular theme associated with carbohydrate synthesis. A brief review of the subject area is provided, but the emphasis in all cases is on describing efficient practical methods to effect the transformations described. Indeed, a number of best synthetic methods are provided within each Chapter. Thus methods will be provided for the synthesis of acceptors, using protecting group chemistry (Chapter 2), as well as entry to, and activation of, a range of popular donors (Chapters 3–5). Modern chemical (Chapters 6 and 7) and enzymatic strategies (Chapter 12) for the assembly of the carbohydrates will also be described. Some specific targets that are either particularly difficult to prepare, or have exceptionally important roles within biological systems, also receive detailed attention. Thus chapters detailing entry to  $\beta$ -linked mannosides (Chapter 8), sialic acid containing carbohydrates (Chapter 9), glycosyl amino acids, of use for accessing glycopeptides, (Chapter 10) and C-linked glycosides (Chapter 11) are presented.

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# Selective Hydroxyl Protection and Deprotection

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## 2.1 INTRODUCTION

In an ideal world it would be possible to effect any desired glycosylation between two free sugars with total control of regio- and stereochemistry to produce a single oligosaccharide. Although enzymes can be sufficiently selective to catalyse such transformations, a general *chemical* solution to this problem is yet to be found. Aside from the problem of controlling the stereochemistry at the newly formed glycosidic linkage, it is simply not possible to select unambiguously, and at will, which hydroxyl group participates in glycosylation and complex mixtures often result.

As in other areas of organic synthesis, ensuring that a particular functional group undergoes reaction without interference from others can be achieved by blocking those others with protecting groups. The general principles of protecting group chemistry and the multitude of methods for their introduction and removal have been summarised in a number of excellent books [1] and review articles [2] but carbohydrates present particular problems as they possess a number of nucleophilic and mildly acidic hydroxyl groups arranged on relatively short carbon chains, and the density of functionality is accordingly high [3]. In general, the various hydroxyl groups cannot be considered in isolation; their reactivity influences, and is influenced by, neighbouring functionality and any modifications made to one of them often leads to changes in the relative reactivity of the others. Because of these subtleties, carbohydrate chemistry may appear to have a large associated ‘lore’ which can seem unnecessarily arcane to the uninitiated; this has led, in the past, to a separation of carbohydrate chemistry from what was regarded as mainstream organic synthesis. Fortunately, the importance of the problems that can be addressed with carbohydrate synthesis, coupled with an extensive and thorough review



literature, has led to a steady integration of the field which now occupies a central position within organic synthesis.

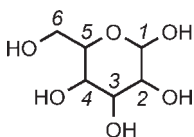
Because of this integration, control of carbohydrate reactivity is increasingly required, not only in the traditional areas of monosaccharide and oligosaccharide synthesis, but also for the preparation of carbohydrate mimics such as *C*-glycosides and imino sugars, and in the synthesis of glycosylated natural products and intermediates for non-carbohydrate compounds.

Capping one or more of the hydroxyl groups reduces the ambiguity in site selectivity during subsequent reactions and judicious choice of protecting group can impart favourable steric and electronic characteristics that may influence reactivity and stereoselectivity. Obviously a protecting group regime needs to be carefully chosen, and this requires many factors to be balanced; of utmost importance are (a) whether a given hydroxyl group can be protected selectively; (b) whether a potential protecting group will survive the conditions of the intended reactions; and (c) whether selective deprotection can be achieved, if required, at the end of the sequence.

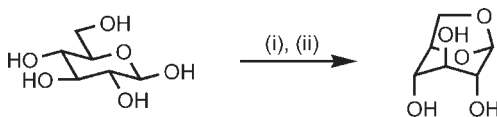
## 2.2 HYDROXYL GROUP REACTIVITY

The issue of the relative reactivity of carbohydrate hydroxyl groups has been the subject of intense investigation for many decades. Comparatively recent developments in chromatographic methods and NMR spectroscopy have allowed reliable product distributions to be assigned from reactions run to low conversion and these results indicate the intrinsic reactivity of particular carbohydrate hydroxyl groups under specified conditions. This information eases the development of protecting group regimes that allow selective masking and unmasking of the various hydroxyl groups as required. Most of the key trends had been identified by the end of the 1970s and this information has been reviewed comprehensively [4].

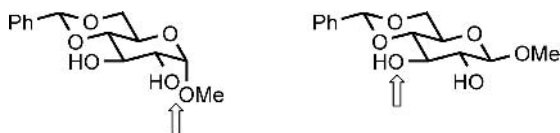
Under acidic conditions, activation of the anomeric (1-) position (Figure 2.1) is straightforward and selective modifications to this centre are easily achieved; as such, protection of the anomeric position will not be specifically discussed in this chapter. Under basic conditions, and in the broadest terms, hexopyranose reactivity follows reasonably consistent lines: the (primary) 6-OH group, being the least hindered, reacts first followed by the (anomeric) 1-OH; of the remaining (secondary) hydroxyls the 2-OH is often the more reactive followed by the 3- and 4-hydroxyls, respectively. Differences in this order reflect the axial/equatorial orientation of



**Figure 2.1** Hexopyranose numbering.



**Figure 2.2** (i) TsCl, pyridine; (ii) aq. NaOH.



**Figure 2.3** Preferred sites for benzylation with BnBr, BaO, DMF.

the hydroxyl groups: axial hydroxyls tend to react more slowly than those in equatorial positions. By forming the 1,6-anhydrosugar, the relative reactivity of the various hydroxyls can be altered because the orientation at each ring position is inverted; Figure 2.2 shows how, in glucose, the three equatorial 2°-hydroxyls become axially disposed in the anhydro sugar [5].

Such generalisations can, however, be misleading as the various hexopyranoses exhibit slightly different reactivity patterns and the anomeric configuration can be critical in determining the site selectivity. In particular, when a (protected) anomeric hydroxyl is *cis* to the 2-OH it is often found that the 2-OH shows enhanced reactivity towards etherification and esterification as exemplified by benzylation of methyl 4,6-*O*-benzylidene  $\alpha$ -D-glucopyranoside under basic conditions (Figure 2.3) [6].

More important are the choice of protecting group and the exact conditions under which it is introduced as these dictate which of the hydroxyl groups reacts. Although the variety of hydroxyl protecting groups [7] can appear bewildering, in practice, the vast majority of carbohydrate syntheses are built around just a handful of tried and tested groups. These are conveniently separated into ethers, esters, silyl ethers, and cyclic acetals (for diol protection).

## 2.3 ETHERS

Simple alkyl groups offer the most reliable form of protection; for example, methyl ethers are stable under aqueous conditions over a wide pH range, are essentially inert to bases, nucleophiles, organometallic reagents, oxidizing and reducing agents, and alkylating agents. In fact, this stability can be problematic in terms of temporary protection as, eventually, the protecting group has to be removed. In the case of methyl ethers the only widely applicable deprotection solution is to employ a combination of Lewis acid and nucleophile, and the conditions may be sufficiently

vigorous to induce unwanted chemistry at other sites in the molecule; in this respect the anomeric centre is especially vulnerable.

For this reason, in complex carbohydrate synthesis, simple ethers are not preferred and, instead, the ethers that have found favour incorporate functionality that can lead to cleavage under rather specific conditions: useful alkyl protecting groups possess good stability under a wide variety of conditions but they have an Achilles' heel that can be targeted when deprotection is needed. Of the ethers the most important are the benzyl (Bn) and allyl (All) groups and their derivatives, the methods for their introduction being roughly equivalent for the two and falling into four classes [8]:

(a) Basic conditions using the appropriate alkyl halide in a polar solvent (or in a relatively non-polar solvent such as THF with an added tetraalkylammonium halide catalyst).

- (i) RBr, NaH, DMF or DMSO; (Method 1)
- (ii) RBr, NaH, THF, cat. Bu<sub>4</sub>Ni; (Method 2)
- (iii) RCl, KOH;
- (iv) RCl or RBr, aqueous NaOH, cat. Bu<sub>4</sub>NHSO<sub>4</sub>;
- (v) RBr, BaO, DMF;
- (vi) RBr, NaH, CuCl<sub>2</sub>, Bu<sub>4</sub>Ni; (Method 3)

(b) Essentially neutral conditions using silver(I) oxide, or via stannyl ethers and stannylene acetals.

- (i) RBr, Ag<sub>2</sub>O, DMF; (Method 4)
- (ii) (Bu<sub>3</sub>Sn)<sub>2</sub>O; then RBr, *N*-methylimidazole;
- (iii) Bu<sub>2</sub>SnO; then RBr, DMF (or RBr, PhCH<sub>3</sub>, cat. Bu<sub>4</sub>NX); (Method 5)

(c) Acidic conditions using the appropriate *O*-alkyltrichloroacetimidate.

- (i) ROC(=NH)CCl<sub>3</sub>, CF<sub>3</sub>SO<sub>3</sub>H; (Method 6)

(d) Methods specific to either allyl or benzyl ethers, see below.

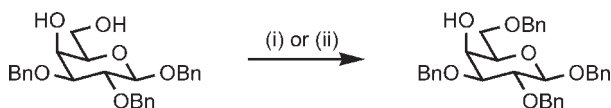
The choice of conditions for alkylation (or any protection method) is dictated by the existing functionality within the carbohydrate. Where there are alternatives within a category, the specific choice is usually made on the basis of previous experience or if the method offers a desired level of selectivity for protection of a particular site. For example, the alkylation conditions within category (a) above [with the exception of (vi)] and that under (c) lead to more or less equivalent levels of selectivity (summarised in section 2.2, Hydroxyl group reactivity) and will not be discussed further. However alkylations and acylations proceeding via *O*-stannyl intermediates (b) and via copper chelates [(a)(vi)] follow set patterns that may differ from these general trends. These alternatives are discussed within sections 2.3.1, Benzyl ethers and 2.3.2, Allyl ethers.

### 2.3.1 Benzyl ethers

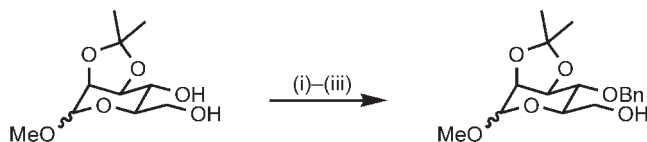
Benzyl ethers are commonly introduced by the conditions in category (a), section 2.3, Ethers, particularly when perbenzylation is required. The choice of conditions does not affect selectivity greatly (Figure 2.4) [9] except when the copper chelate method is employed. In the latter method dialkoxides (produced from diols with NaH) are deactivated by regioselective complexation of a copper salt so that the free alkoxide reacts preferentially; Figure 2.5 illustrates a typical case [10] which should be compared with the variants in Figure 2.4.

The use of stannylene acetals and stannyl ethers is widely used for achieving selective benzylation, the selectivity paralleling that observed for allylation (see section 2.3.2, Allyl ethers).

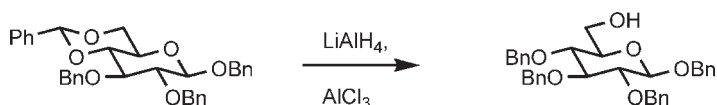
Divergent selectivity in obtaining benzyl-protected sugars can be achieved by Lewis acid mediated reduction of benzylidene acetals (Methods 7 and 8). Figure 2.6 illustrates selective release of the 6-OH using the combination  $\text{LiAlH}_4/\text{AlCl}_3$  in which the comparatively bulky Lewis acid initiates oxonium ion formation by complexing to the sterically more available 1°-benzylidene acetal oxygen [11]. Conversely (Figure 2.7) use of protic acid in combination with  $\text{NaBH}_3\text{CN}$  leads to ring-opening in the opposite sense via protonation of the slightly more basic 2°-acetal oxygen [12]. Interestingly, the regiochemistry in the cleavage of benzylidene derivatives of 1,2-diols (see section 2.6, Acetals) is dependent on the stereochemistry at the benzylic carbon (Figure 2.8); the enhanced basicity of dioxolane oxygens compared to dioxane oxygens is apparent in this example [13].



**Figure 2.4** (i)  $\text{BnCl}$ ,  $\text{NaH}$ ,  $\text{DMF}$ ; (ii)  $\text{BnBr}$ , 5% aq.  $\text{NaOH}$ , cat.  $\text{Bu}_4\text{NHSO}_4$ .



**Figure 2.5** (i)  $\text{NaH}$  (2.0 equiv.); (ii)  $\text{CuCl}_2$ ; (iii)  $\text{BnI}$ .



**Figure 2.6**

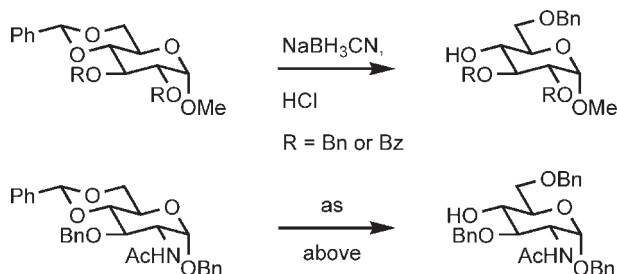


Figure 2.7

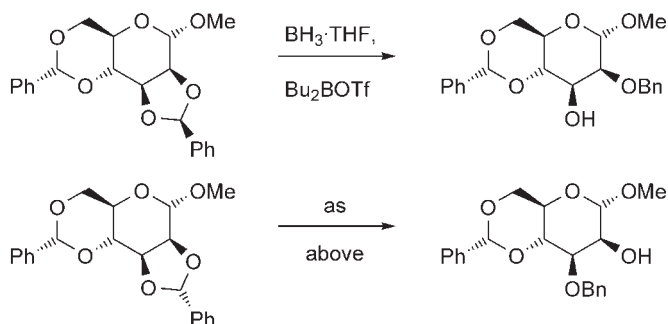
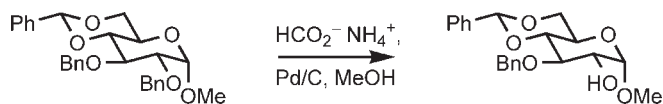
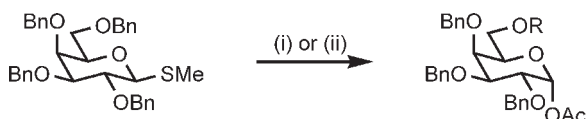
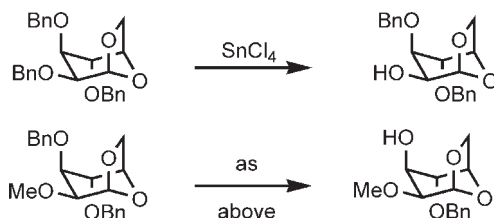


Figure 2.8

Whilst benzyl ethers are stable to a wide range of reaction conditions they may be cleaved selectively by reduction, usually by Pd-catalysed hydrogenolysis [14] (Method 9) (use of Pt can lead to hydrogenation of the benzene ring [15]), transfer hydrogenolysis [16], or under dissolving-metal conditions [17]. However, the benzyl group is *so* widely used in synthesis that many specialised alternatives have been found. Unsurprisingly, conditions that cleave methyl ethers can lead to benzyl ether deprotection, and the enhanced reactivity of the benzylic methylene group can be exploited by oxidizing agents, particularly in cases where the phenyl ring bears electron-donating substituents.

Selectivity in the partial deprotection of a multi-benzylated carbohydrate can be achieved but is often unpredictable; if high selectivity is required for the exposure of a specific hydroxyl group it is usually more effective to choose the protecting groups carefully at the outset. However, transfer hydrogenolysis can be regioselective (Figure 2.9) [18], and indirect deprotection by acetolysis has been widely used [19]. The examples in Figure 2.10 illustrate the general preference for acetolysis of 1°-benzyl ethers, the anomeric thiomethyl group being rapidly activated under these reaction conditions [20].

Under Lewis acidic conditions deprotection has been shown [21] to be selective when complexation by three oxygen atoms is possible, as in the anhydromannose examples in Figure 2.11 in which deprotection favours 2-OBn > 3-OBn > 4-OBn;

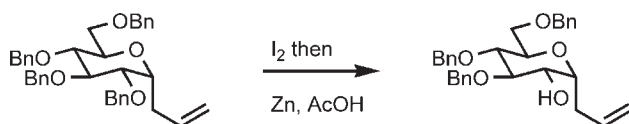
**Figure 2.9****Figure 2.10** (i)  $\text{Ac}_2\text{O}$ , cat.  $\text{I}_2$ ,  $0^\circ\text{C}$ , 5 min ( $\text{R} = \text{Bn}$ ); (ii)  $\text{Ac}_2\text{O}$ , cat.  $\text{I}_2$ ,  $0^\circ\text{C}$ , 1 h ( $\text{R} = \text{Ac}$ ).**Figure 2.11**

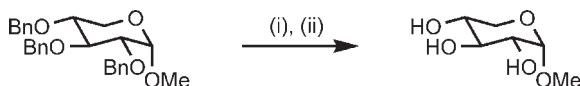
1,6-anhydroglucose analogues, which lack the required disposition of oxygens, fail to react with useful selectivity.

Intramolecular activation of benzyl ethers can lead to unexpected but highly selective deprotection. In the example in Figure 2.12 [22], the proximity of the 2-oxygen to the allyl group led to its capture by the intermediate iodonium ion and loss of benzyl as benzyl iodide; reductive elimination of the so-formed iodomethyl tetrahydrofuran completed the process which was used as a key step during a synthesis of mannosamine and glucosamine C-glycosides.

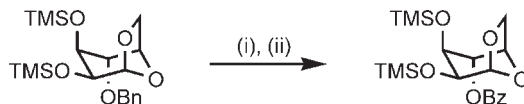
Finally, benzylic oxidation offers a third method for deprotection, as an alternative to hydrogenolysis or electrophilic activation. For example, ozone converts benzyl ethers into benzoate esters at around  $0^\circ\text{C}$  (Figure 2.13) [23], and ruthenium tetroxide generated *in situ* has been used for the same purpose; the conditions are sufficiently mild that labile trimethylsilyl ethers remain intact (Figure 2.14) [24].

A great attraction of benzyl as a protecting group core is its ability to be ‘tuned’ both sterically and electronically which leads to a degree of graduated lability within

**Figure 2.12**



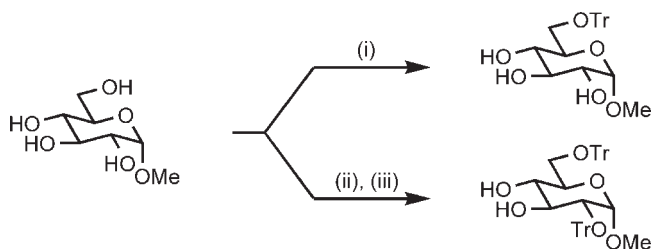
**Figure 2.13** (i)  $\text{O}_3$ ; (ii)  $\text{NaOMe}$ ,  $\text{MeOH}$ .



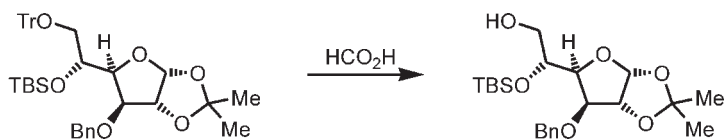
**Figure 2.14** (i)  $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ ,  $\text{NaIO}_4$ ; (ii)  $\text{NaIO}_4$ .

the class. Thus, for added selectivity during protection, triphenylmethyl (trityl, Tr) and, less commonly, diphenylmethyl (benzhydryl) ethers are employed; these bulky protecting groups provide a steric shield around the protected alcohol and the extra phenyl rings lead to an increased tendency to ionize under solvating ( $\text{S}_{\text{N}}1$ ) conditions, see below. The trityl group has been traditionally employed for selective protection of the (primary) 6-OH in hexopyranoses, usually using  $\text{TrCl}$  in pyridine or similar, e.g. Figure 2.15 [25]. Tritylation of the 2°-hydroxyls is also possible and the second example in Figure 2.15 illustrates the enhanced reactivity of the 2-OH in methyl  $\alpha$ -D-glucopyranoside in forming the 2,6-bis(tributylstannyl) ether prior to tritylation [26]. When no longer required, trityl ethers may be cleaved under very mild protic or Lewis acidic conditions (as hydrogenolysis can be unreliable) that leave potentially acid-labile protecting groups intact (Figure 2.16) [27].

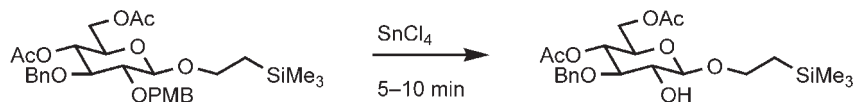
In terms of *electronic* tuning, it has been mentioned already that electron-donating phenyl substituents confer increased lability towards oxidizing agents, and *para*-methoxybenzyl (PMB) ethers—introduced by methods analogous to those used for the benzyl group—are routinely employed because they can be removed highly



**Figure 2.15** (i)  $\text{TrCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{DMAP}$ ; (ii)  $\text{Bu}_6\text{Sn}_2\text{O}$ ; (iii)  $\text{TrCl}$ .



**Figure 2.16**

**Figure 2.17**

selectively by one-electron oxidants such as DDQ [28] or CAN [29] (in addition to the methods that cleave simple benzyl ethers, e.g. Figure 2.17) [30]. As expected, 3,4-dimethoxybenzyl (DMPM) ethers are more prone to oxidative deprotection and, for example, high selectivity can be achieved between PMB (= MPM) and DMP ethers with DDQ [28].

Finally, the cleavage specificity of the benzyl group can be modified by incorporating molecular triggers; for example, *ortho*-nitrobenzyl (ONB) ethers may be cleaved in the presence of most other protecting groups by photolysis as shown in spectacular fashion in the last stages of a solid-phase synthesis of heptasaccharide phytoalexin elicitor (HPE) (Figure 2.18) [31].

### 2.3.2 Allyl ethers

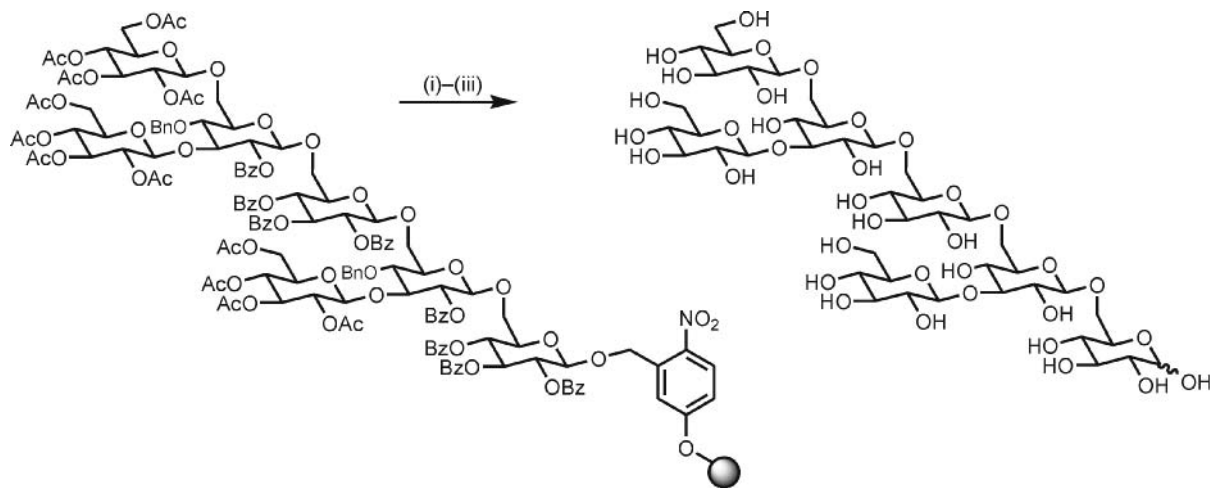
Second only to benzyl derivatives are allyl (All) ethers because they are easy to introduce, survive most of the methods for glycoside formation, yet are readily removed by isomerisation (by strong base or transition metal catalysis) and hydrolysis, or under oxidative conditions that leave most other groups intact. The use of allyl protecting groups has been reviewed [32]. Allyl protection offers an opportunity to discuss alkylation via stannylene acetals as this is a favoured method for achieving selectivity in complex systems. The method is particularly suited for the mono-protection of 1,2-diols via prior formation of five-membered ring stannylene acetals with  $\text{Bu}_2\text{SnO}$  and the following general trends have been observed:

- Stannylene acetals formed from a 1°, 2°-diol lead to preferential alkylation of the 1°-hydroxyl;
- Alkylation of the equatorial hydroxyl is preferred from a 2°-equatorial, 2°-axial 1,2-diol;
- *Cis*-1,2-diols are preferentially alkylated (at the equatorial hydroxyl) over *trans*-1,2-diols;
- Equatorial, equatorial 1,2-diols show little selectivity unless one of the oxygens is *cis*- to a third oxygen atom (e.g. in  $\alpha$ -glycosides).

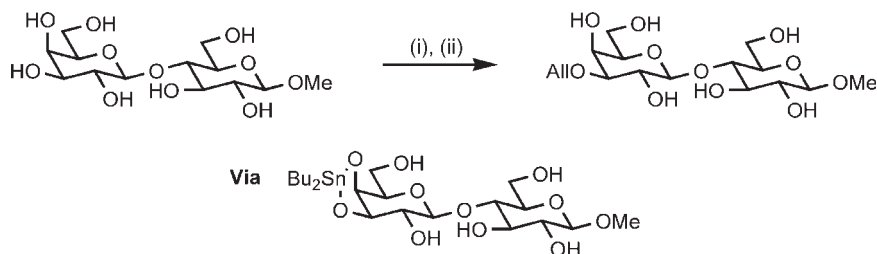
From these trends, which apply equally to benzyl and allyl protection, the highly selective allylation of just one out of seven hydroxyls in the example in Figure 2.19 is explainable since this is the only equatorial hydroxyl of a *cis*-1,2-diol pair [33].

A feature specific to allyl protection is the possibility of introducing the allyl group indirectly via the allyl carbonate by Pd-mediated decarboxylation. This has





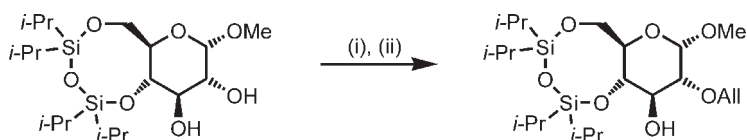
**Figure 2.18** (i)  $h\nu$ ; (ii) NaOMe, MeOH; (iii)  $\text{H}_2$ , cat. Pd/C.



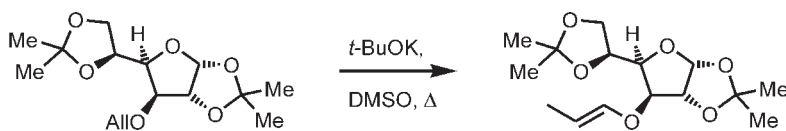
**Figure 2.19** (i)  $\text{Bu}_2\text{SnO}$ ; (ii)  $\text{AlI}_3$ ,  $\text{Bu}_4\text{NBr}$ .

the advantage over traditional alkylation methods that base-sensitive groups are tolerated because the first (acylation) step can be achieved under very mild conditions—allyl chloroformate and pyridine—and the second step is essentially neutral, e.g. Figure 2.20 [34]. Note, again, the enhanced reactivity of the 2-OH in this example. A useful one-step variant is described in Method 10.

Deprotection of allyl ethers is usually effected by base-mediated isomerisation of the allyl double-bond to produce the corresponding enol ether, e.g. Figure 2.21 [35] that can then be readily hydrolysed under conditions that leave most acid-sensitive functionality intact. Transition metal catalysts offer an alternative to bases for the initial isomerisation; these reagents offer the possibility of isomerisation and hydrolysis in a one-pot process (Method 11). Selectivity between different types of allyl protecting group is possible as the example in Figure 2.22 shows [36].



**Figure 2.20** (i)  $\text{ClCO}_2\text{All}$ , pyridine; (ii) cat.  $\text{Pd}(\text{PPh}_3)_4$ .



**Figure 2.21**

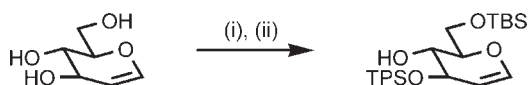


**Figure 2.22** (i) Ir(COD)(PMePh<sub>2</sub>)<sub>2</sub>·HPF<sub>6</sub> (activated with H<sub>2</sub>); (ii) HgO, HgCl<sub>2</sub>, aqueous acetone.

### 2.3.3 Silyl ethers

The discovery of silyl protecting groups for alcohols, and silylenes and related derivatives for diol protection, has given carbohydrate chemists convenient alternatives to the classical trityl protecting group for selective blocking of the least hindered hydroxyl, and to 1,3-diol protection with benzylidene. In the first case, bulky silyl ethers such as *tert*-butyldimethylsilyl (TBDMS, TBS) and *tert*-butyldiphenylsilyl (TBDPS) are readily and cleanly introduced onto the 1°-hydroxyl (Method 12) with any 2°-hydroxyls being silylated in order of steric availability (Figures 2.23 [37] and 2.24 [38]) and with the usual preference of equatorial before axial (Figure 2.25) [39]. The trialkylchlorosilane in the presence of a base (usually imidazole in DMF) is generally sufficient, more hindered situations often call for the more reactive silyl triflates. The use of silver nitrate in these reactions increases the selectivity for silylation of 1°-hydroxyl groups, but adding pyridine to the mixture allows the 2°-hydroxyls to be silylated as well.

A characteristic, and often problematic, feature of silyl protecting groups is their tendency to migrate to proximal hydroxyl functionality, particularly under basic conditions. Whilst this sometimes leads to unexpected results, e.g. Figure 2.26 [40] as long as the possibility is appreciated, appropriately mild conditions can be employed to minimize, if not avoid, this.



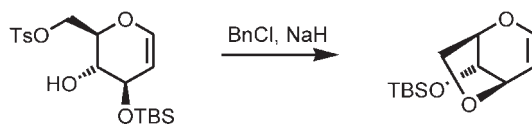
**Figure 2.23** (i) TBSCl, imidazole; (ii) TPSCl (= Ph<sub>3</sub>SiCl), imidazole.



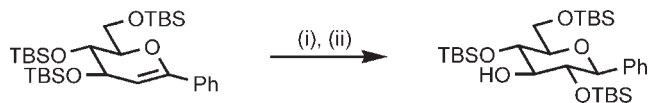
**Figure 2.24**



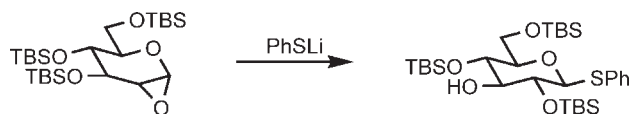
**Figure 2.25**



**Figure 2.26**



**Figure 2.27** (i)  $\text{BH}_3$ ; (ii)  $\text{H}_2\text{O}_2$ , aq.  $\text{NaOH}$ .

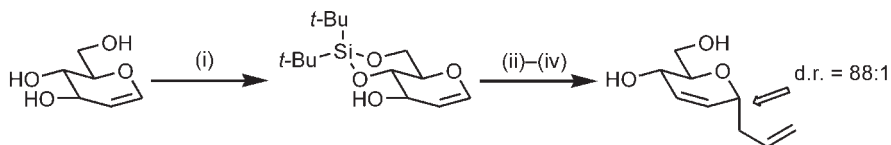


**Figure 2.28**

In general, silyl migrations tend to result in statistical mixtures of regioisomeric silyl ethers but, in the context of carbohydrates, a single silyl ether may predominate; this is usually the case when migration relieves steric crowding as in the examples in Figures 2.27 [41] and 2.28 [42] in which the 3-OH group is revealed after silyl migration to the newly formed 2-OH in each case. In the example in Figure 2.27 silyl migration could be avoided by running the oxidation step in the presence of a pH 7 phosphate buffer.

Two types of silyl protecting groups for diols are in widespread current use, their particular structures stemming from the known high susceptibility of cyclic dialkoxysilanes towards solvolysis (and attack by nucleophiles in general), particularly when the ring is five- or six-membered. Firstly, nucleophilic attack can be inhibited with bulky *tert*-butyl substituents, and di-*tert*-butylsilylenes form an excellent alternative to benzylidene protection of the 4,6-diol pair in hexopyranoses (Figure 2.29) [43]. Because the method requires the silyl ditriflate, scrupulously anhydrous conditions are necessary for good yields. The example illustrates the inertness of this group to Lewis acidic conditions and its selective cleavage by  $\text{F}^-$  ion delivered as tetrabutylammonium fluoride (TBAF).

Secondly, the diol may be encompassed in a seven- or eight-membered ring where the lessened ring strain reduces hydrolytic lability. These tetraisopropylidisiloxanylidene (TIPDS) derivatives initiate protection, as expected under kinetic conditions, by reaction at the least hindered ( $1^\circ$ -if present) hydroxyl to give similar overall protection to the silylenes discussed above (Method 13). In suitable cases, these first-formed derivatives may be isomerized under acid catalysis (in DMF) to release the  $1^\circ$ -OH and tie up two  $2^\circ$ -hydroxyls, e.g. Figure 2.30 [44].



**Figure 2.29** (i)  $t\text{-Bu}_2\text{Si}(\text{OTf})_2$ , pyridine; (ii)  $\text{Ac}_2\text{O}$ , pyridine; (iii) allyl- $\text{SiMe}_3$ ,  $\text{TMSOTf}$ ; (iv) TBAF.

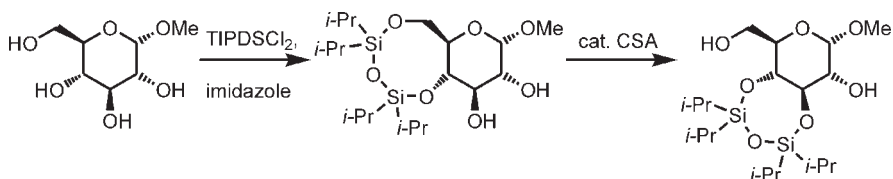


Figure 2.30

Silyl ethers are renowned, and used, for their highly selective deprotection with fluoride sources (Method 14) as mentioned above, but selecting between different silyl ethers under these conditions is not always reliable unless the silyl alkyl substituents are sufficiently different. However, deprotection of silyl ethers of 1°-alcohols, in the presence of similarly protected 2°-alcohols, is routinely possible under either acidic or basic conditions [45].

## 2.4 ESTERS

Ester protecting groups are ubiquitous in carbohydrate chemistry because acylation of the hydroxyl functions is *such* an efficient process in general that overall polyacylation proceeds reliably and in high yields (Method 15). Acylation is generally effected under standard conditions—the appropriate acyl chloride or acid anhydride in the presence of, for example, pyridine, sometimes in the presence of efficient nucleophilic catalysts such as DMAP or HOBt—and provides effective electronic deactivation of the nucleophilicity of protected oxygens by conjugation. In general, esters are moderately stable under acidic conditions (in the absence of water and other hydroxylic solvents) but are labile in the presence of nucleophiles, particularly alkoxides, amines, organometallics, and hydride transfer agents. This is useful in the context of carbohydrate synthesis since acetal-based protecting groups and glycosidic linkages remain unaffected by the preferred reagents for ester deprotection. The great versatility of esters, though, is the degree of graduation of lability within the class and, amongst the most commonly used esters, there is a huge range of reactivity available. The relative rates for the saponification of substituted acetates (Figure 2.31) reflect the effect of electron-withdrawing groups on the ease of nucleophilic addition to the carbonyl carbon [46].

As well as offering electronic deactivation of the nucleophilicity of the oxygens, the use of bulky esters adds further elements of control: (i) bulky

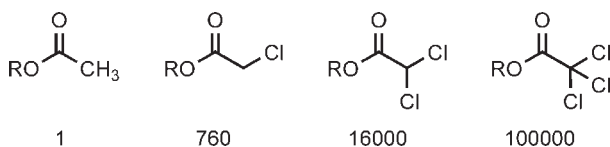


Figure 2.31

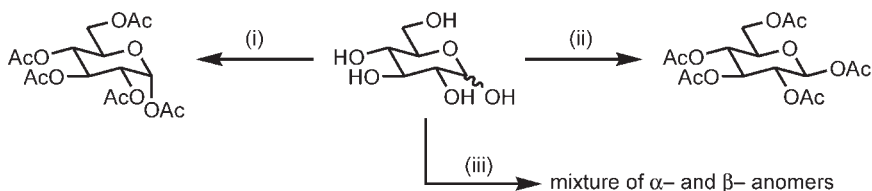
acylating agents are usually more discriminating which enables, for example, selective acylation of the (primary) 6-OH group in hexopyranoses; (ii) once introduced, a sterically demanding ester provides a local encumbrance to approaching reagents such that chemistry is directed to sites physically removed from the first-protected position.

With these features in mind the prevalence of acetate, chloroacetate, benzoate (and substituted variants), and pivaloate esters in carbohydrate hydroxyl protection can be readily understood.

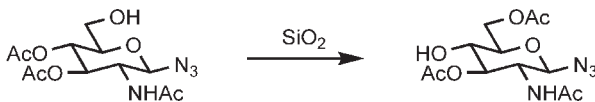
### 2.4.1 Acetates

Acetates are the most commonly used of the esters for hydroxyl protection, particularly for peracylation (Figure 2.32) [47]; although selective partial acetylation can be achieved, the tendency of acetates to migrate can lead to a loss in observed selectivity. In some cases, as with silyl ethers, migration can be sufficiently reliable to be synthetically useful; for example, acetate migration can be used efficiently to lead to simultaneous release of the 4-OH and protection of the 6-OH in glucose derivatives (Figure 2.33) [48].

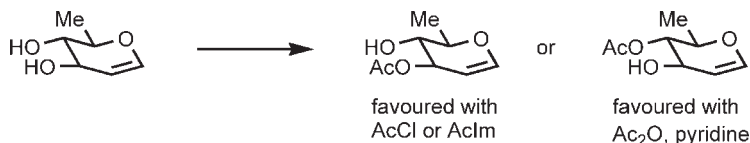
Achieving *selective* acetylation by standard methods tends to be unreliable and reagent specific (as exemplified in Figure 2.34) [49], however, reliable selectivity can be achieved via the prior preparation of stannylene acetal intermediates (see section 2.3.2, Allyl ethers) or, complementarily, via orthoesters derived from 1,2-*cis*-diol pairings (Figure 2.35) [50].



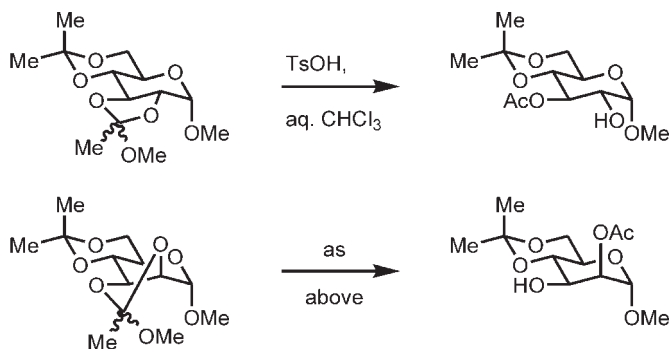
**Figure 2.32** (i)  $\text{Ac}_2\text{O}$ ,  $\text{ZnCl}_2$ ; (ii)  $\text{Ac}_2\text{O}$ ,  $\text{NaOAc}$ ; (iii)  $\text{Ac}_2\text{O}$ , pyridine.



**Figure 2.33**

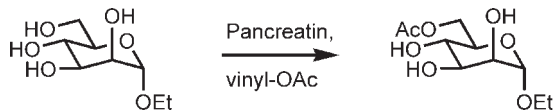
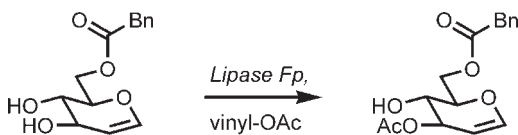
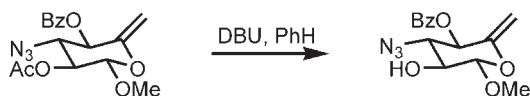


**Figure 2.34**

**Figure 2.35**

Additionally, extensive investigation of lipases and related enzymes in the manipulation of acetates has led to some extremely selective procedures [51]. For example, monoacetylation of the 6-OH in hexopyranoses is possible (Figure 2.36) [52] and fine discrimination between the 2°-hydroxyls can be achieved (Figure 2.37) [53].

Once the acetates have served their purpose their removal is most easily effected by the Zemplén procedure [54] (catalytic NaOMe in MeOH) (Methods 16 and 17) but more selective methods—such as guanidine in ethanol [55], or DBU in benzene (Figure 2.38) [56]—can leave less reactive benzoate esters intact. For deprotection, lipases can again offer exquisite discrimination between acetates which can be particularly useful in sensitive substrates where classical methods fail. An idea of the selectivity that can be attained is given in Figure 2.39 which shows the specific sites of deacetylation in peracetyl sucrose using lipases from different organisms [57].

**Figure 2.36****Figure 2.37****Figure 2.38**

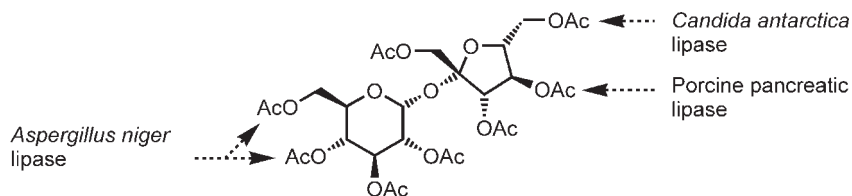


Figure 2.39

## 2.4.2 Substituted acetates

As mentioned, tuning of reactivity within esters is possible by modifying the electrophilicity of the ester carbonyl (Figure 2.31). Amongst the modified acetates, the chloroacetyl (ClAc) group has found most favour as it is sufficiently reactive to be cleavable in the presence of unadorned acetates and benzoates under standard solvolysis conditions, e.g. Figure 2.40 [58], or with hydrazine, e.g. Figure 2.41 [59]. In addition, the  $\alpha$ -chloro substituent is reactive towards nucleophilic substitution by soft nucleophiles and in the case of, for example, thiourea this is followed by cyclisation and expulsion of the free hydroxyl as in Figure 2.42 [60].

Moving in the other direction, pivaloate (Pv, Piv) esters are heavily deactivated with respect to acetates which results in high selectivity during esterification and low reactivity towards the conditions routinely used for acetate and benzoate

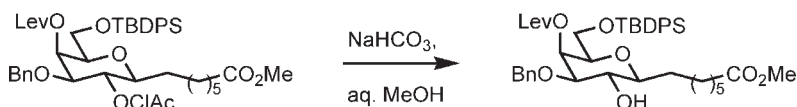


Figure 2.40

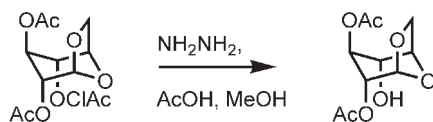


Figure 2.41

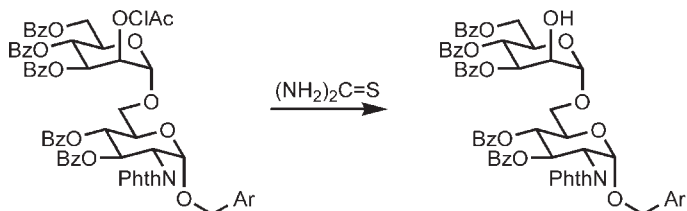


Figure 2.42



deprotection. The selectivity of pivaloylation can be relied upon as a means for capping 1°-hydroxyls in the presence of 2°-hydroxyls and this is one of the methods of choice for 6-OH protection in hexopyranoses; in the example shown in Figure 2.43 the use of excess acylating agent reveals the enhanced reactivity of the 2-OH in  $\alpha$ -D-glucopyranosides with the 2,6-dipivaloate being obtained as the major product [61].

Whilst Zemplén deprotection of Piv esters works well in simple cases, the cleavage can be sufficiently difficult that other base-labile protecting groups (such as silyl ethers) fail to survive and, in these situations, aprotic conditions are needed. Hydride transfer reagents are usually employed to solve this problem as shown in Figure 2.44 for a key step during the synthesis of iminolyxitsols; note that the dibenzylidene imine functionality is also reduced under these conditions [62].

Certain functionalised esters combine the virtues of acyl protection (ease of introduction, electronic deactivation, lability to base) with the ability to be deprotected under specialised conditions that leave other esters unaffected. Of these, the only example that has seen reasonably widespread application is the levulinate (Lev) derivative which, in chemistry that parallels the cleavage of ClAc derivatives with soft nucleophiles, are cleavable by conversion of the comparatively reactive ketone functionality into a nucleophilic entity that can cyclise onto the ester carbonyl releasing the protected hydroxyl. The example in Figure 2.45 shows that the acetates survive unscathed in Lev deprotection with hydrazine so that only the 2-OH is released [63].

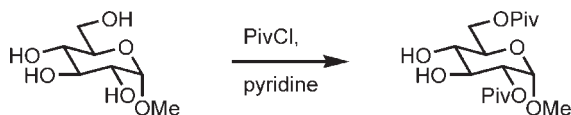


Figure 2.43

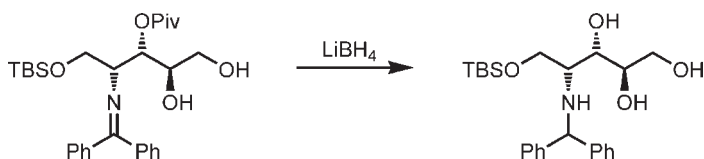


Figure 2.44

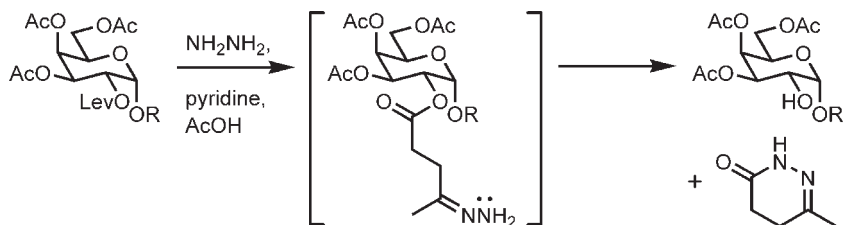


Figure 2.45

### 2.4.3 Benzoates

Stabilization of the ester carbonyl group by conjugation with the phenyl ring in benzoate esters confers an increase in acylation selectivity, a reduced tendency to migrate, and an increased stability towards nucleophiles. These features have placed benzoate (Bz) protection in a central position as a strategy for carbohydrate synthesis, second only to acetates in popularity. Introduction of benzoates follows methods analogous to those used for acetylation and benzylation via stannylene acetals provides useful levels of selectivity in discriminating between *cis*-1,2- and 1,3-diol pairs (Figures 2.46 [64] and 2.47 [65], cf. section 2.3.2, Allyl ethers).

As with acetates, benzoate migration can be used to achieve selective protection and release in ways inaccessible by direct methods. Thus, in Figure 2.48, benzylation took place kinetically at the 3-OH group leaving the 2-OH free, but alkaline isomerisation led to benzoate migration to the 2-position leaving the 3-OH free for further manipulation [66].

Finally, because benzoic acid is an excellent partner in the Mitsunobu reaction, benzoate formation with inversion has been used to prepare protected variants of relatively rare carbohydrates from readily available precursors. Thus, Mitsunobu esterification at the 3- and 6-positions in methyl  $\beta$ -D-glucopyranoside proceeded with inversion of the C-3 configuration to provide an efficient synthesis of methyl  $\beta$ -D-allopyranoside after Zemplén methanolysis (Figure 2.49). The  $\alpha$ -anomer was reactive only at the 6-position (to maintain the gluco-configuration) under the same conditions [67].

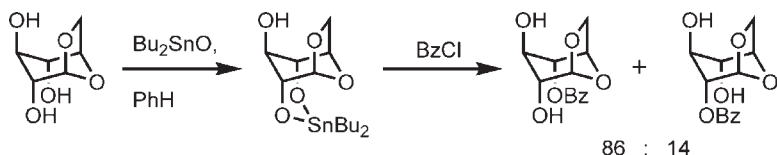


Figure 2.46

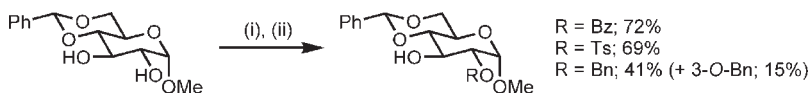


Figure 2.47 (i)  $\text{Bu}_2\text{Sn}(\text{OMe})_2$ ; (ii)  $\text{BzCl}$  or  $\text{TsCl}$ ,  $\text{Et}_3\text{N}$  or  $\text{BnBr}$ ,  $\text{Bu}_4\text{NI}$ .

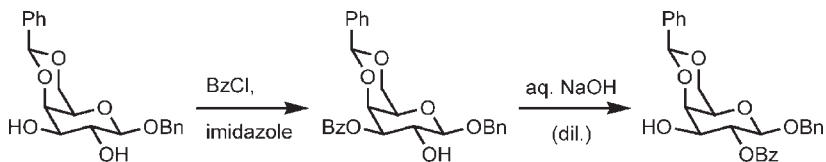
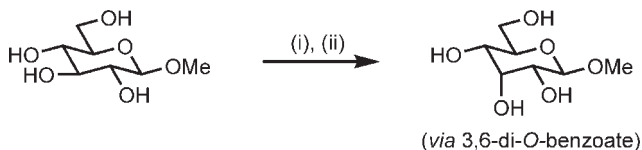


Figure 2.48



**Figure 2.49** (i)  $\text{PPh}_3$ , DEAD,  $\text{BzOH}$ ; (ii)  $\text{NaOMe}$ ,  $\text{MeOH}$ .

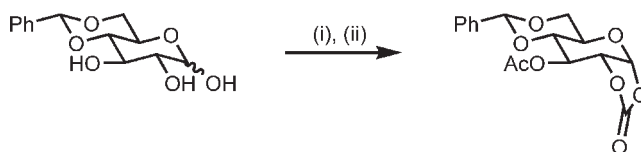
## 2.5 CARBONATES

Alkyl carbonate derivatives greatly extend the versatility of acyl protection since the alkyl residue can be chosen to provide derivatives that are cleavable under highly specific conditions as tabulated below.

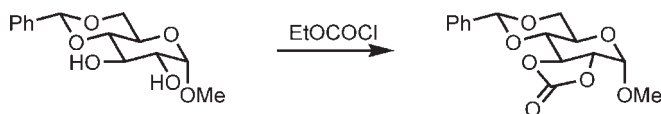
Derivative	Abbreviation	Typical deprotection conditions
Allyloxycarbonyl	Alloc	$\text{Pd}(0) + \text{nucleophile}$
2-(Trimethylsilyl)ethyl oxycarbonyl	Tmsec, Teoc	$\text{F}^-$ sources
9-Fluorenylmethyloxycarbonyl	Fmoc	Piperidine
<i>tert</i> -Butyloxycarbonyl	Boc	TFA
2,2,2-Trichloroethyloxycarbonyl	Troc	$\text{Zn}$ , $\text{AcOH}$
Benzyloxycarbonyl	Cbz, Z	$\text{H}_2$ , $\text{Pd}$ catalyst

Although these derivatives offer great potential for highly site specific manipulations, their use in carbohydrate chemistry has been somewhat limited mainly because the procedures and strategies based on the classical protecting groups are tried and tested. Of these, probably the widest use has been made of Alloc protection because this provides a convenient route to allyl ethers (see section 2.3.2, Allyl ethers).

More widely used are cyclic carbonates derived from 1,2-diols, which form most readily between diols bearing a *cis*-relationship (Figure 2.50) [68] although *trans*-1,2-diols can also be mutually protected in this way (Figure 2.51) [69] as can *cis*-1,3-



**Figure 2.50** (i)  $\text{Im}_2\text{CO}$ ; (ii)  $\text{Ac}_2\text{O}$ .



**Figure 2.51**

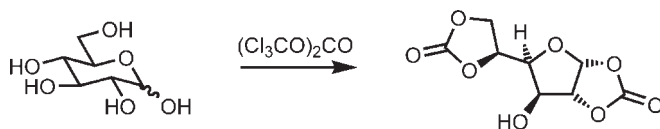


Figure 2.52

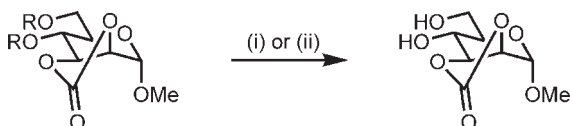
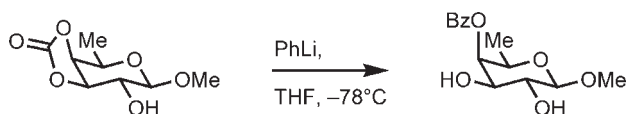
Figure 2.53 (i)  $\text{R} = \text{Cbz}$ ;  $\text{H}_2$ ,  $\text{Pd/C}$ ; (ii)  $\text{R,R} = \text{benzylidene}$ ; aq.  $\text{HCl}$ , acetone.

Figure 2.54

diols provided the hydroxyls are sufficiently close. The preference for 1,2-diol pairings leads to protection of, for example, glucose in its furanose form (cf. acetonide formation, section 2.6, Acetals) (Figure 2.52) [70].

Although all carbonates may be cleaved with varying ease under basic solvolysis conditions, selective cleavage of a particular type of carbonate in the presence of others can be achieved by the side-chain specific methods tabulated above. Carbonate hydrolysis under acidic conditions is usually very slow and hydrolysis of, for example, benzylidene derivatives proceeds without competitive cleavage of the carbonate; both these features are illustrated in Figure 2.53 [71].

Protecting group interchange is also possible from carbonates and, for example, cyclic carbonates of 1,2-diols may be treated with organolithium reagents at low temperature to effect a partial deprotection wherein the less hindered hydroxyl remains protected as an ester (Figure 2.54) [72].

## 2.6 ACETALS

Cyclic carbonates provide one method for the mutual protection of 1,2- and 1,3-diols within an acid-stable group, but diol protection within acetals (acid-sensitive) is of far greater importance. The reaction between acetone and D-fructose was explored by Fischer towards the end of the 19th century [73] and the general method remains one of the most important for blocking of pairs of hydroxyls. Although acetal formation was classically, and still is usually, run under equilibrating conditions that give rise to the most stable product, more recent procedures that operate under

kinetic control have been introduced that add to the versatility of this protecting group type. In the context of carbohydrate protection, two acetal derivatives dominate: benzylidene and isopropylidene (acetone). The two are complementary both in terms of their site selectivity and of their deprotection chemistry, and the choice of which to use, therefore, is normally quite clear.

Under ‘classical’ conditions (acid-catalysed [74] condensation of the sugar with either acetone or benzaldehyde with removal of water) the reactions are subject to thermodynamic control and the major products can be rationalised on stability grounds. Given a choice of 1,2- or 1,3-diol protection, benzylidenes protect the 1,3-pair because of the greater stability of dioxanes over dioxolanes, and isopropylidenes protect the 1,2-pair (particularly if these are part of an acyclic chain or are *cis*-disposed on a furanose or pyranose ring) as the resulting dioxolanes do not suffer from the 1,3-diaxial interactions that are necessarily present in dioxanes (Figure 2.55). This preference is graphically illustrated by the well-known reactions of glucose with either acetone (Method 18) or benzaldehyde; in the former case the most stable product derives from the furanose form since this presents two potential 1,2-diol pairs whereas the pyranose form can only form one (the other would have to include a relatively unstable *trans*-fused isopropylidene) (Figure 2.56) [75]. Obviously, if the maximum number of diol pairs can be protected in the pyranose form then this form of the sugar will remain in the protected version as shown in the galactopyranose example (Figure 2.57) [76].

As an alternative to direct condensation with either acetone or benzaldehyde, transacetalation with 2,2-dimethoxypropane (Method 19) or  $\alpha,\alpha$ -dimethoxytoluene (Method 20) is popular because, with DMF as solvent and CSA as the catalyst,

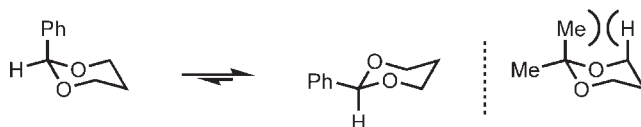


Figure 2.55

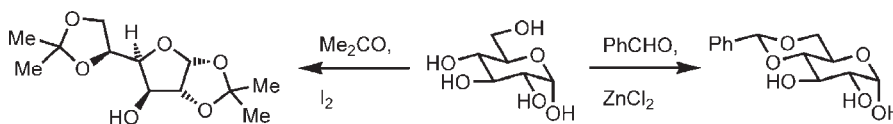


Figure 2.56

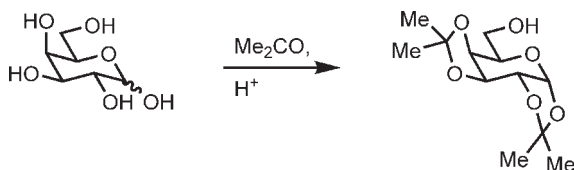


Figure 2.57

liberated methanol can be distilled off under slight vacuum in order to drive the equilibrium process to completion. With 2,2-dimethoxypropane and glucose this procedure gives rise to the 'kinetic' product, i.e. the 4,6-*O*-isopropylidene derivative, that derives from initial reaction at the (least hindered) 6-OH. Kinetic isopropylidene formation can also be achieved with methyl isopropenyl ether catalysed by TsOH (Figure 2.58) [77] and, more recently, by oxidative catalysts such as DDQ [78] and CAN [79] which allow the protection of acid-sensitive substrates such as D-glucal (Figure 2.59). Acetal derivatives may also be prepared under non-acidic conditions using the appropriate 1,1-dihalide in the presence of a base (Figure 2.60) [80].

Once in place, both isopropylidene and benzylidene functionality survive many routine synthetic operations and, when no longer required, may be readily removed by hydrolysis. Two aspects of acetal deprotection are of interest here: (1) partial hydrolysis of bis-acetals, particularly bis(isopropylidenes); and (2) protecting group interchange, particularly of benzylidenes.

A number of steric and electronic features underpin the selectivity of acetal hydrolysis but, in this context, it is just the results that are important: (1) *trans*-dioxolanes are more readily hydrolysed than dioxanes than are *cis*-dioxolanes; (2) acetals derived from a diol in which one of the hydroxyls is primary are hydrolysed more rapidly than those in which both are secondary, particularly if, in the latter

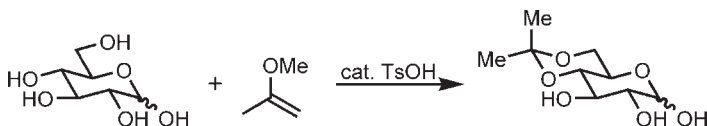


Figure 2.58

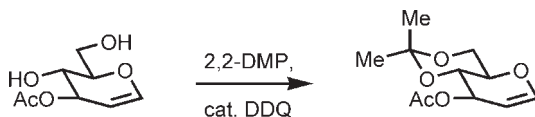
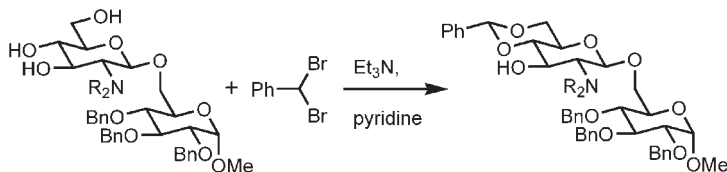


Figure 2.59



R<sub>2</sub>N- = 2,5-dimethylpyrrol-1-yl

Figure 2.60

case, one of the oxygens is anomeric. In principle, this means that benzylidene acetals should be (and are) cleavable in preference to typical isopropylidenes but, in practice, it is usually more straightforward to take advantage of the lability of the former towards catalytic hydrogenolysis (Pd or Pd/C). The second aspect is manifested in the much used selective hydrolysis of the 5,6-*O*-isopropylidene in diacetone glucose [81] and the idea was used to free the side-chain hydroxyls as a key step in a synthesis of substituted cyclopentanes (Figure 2.61) [82].

Selective reduction of benzylidene acetals to form mono-benzyl protected diols was discussed in section 2.3.1, Benzyl ethers but differentiation of the oxygen atoms within benzylidene derivatives can also be achieved in the widely used Hanessian procedure. In this process, benzylic bromination, oxonium ion formation, and nucleophilic attack by bromide ion at the (1°-) 6-position leads to the 4-*O*-benzoyl-6-deoxy-6-bromo derivative, Figure 2.62 [83].

Benzylidene and isopropylidene derivatives provide an effective general method for protecting 1,3-diols and *cis*-1,2-diols but, until recently, no similarly general procedure was available for selecting out 1,2-diols bearing a *trans*-diequatorial relationship. Fortunately, an elegant solution to this problem has been developed that exploits steric and stereoelectronic control during the thermodynamically driven formation of 1,2-bis-acetals from 1,2-diketones and their synthetic equivalents [84]. For example, butanedione selects just the 2,3- and 3,4-diol pairs in galactopyranose and mannopyranose (Method 21) rings, respectively, because the ‘butanediactal’ (BDA) products form a stable *trans*-decalin-like structure with the methyl groups equatorial, and the number of anomeric effects maximised (Figure 2.63) [85].

This chemistry has gained rapid acceptance and variants based on cyclohexanediactal (CDA derivatives) and dispiroketal (‘dispoke’) have been applied to efficient syntheses of oligosaccharides [86].

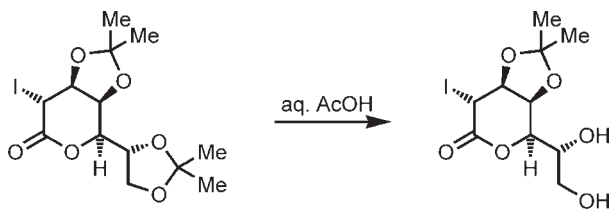


Figure 2.61

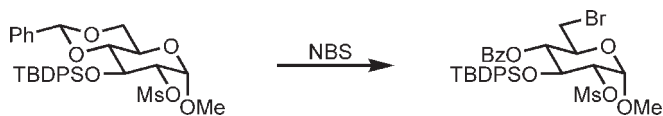


Figure 2.62

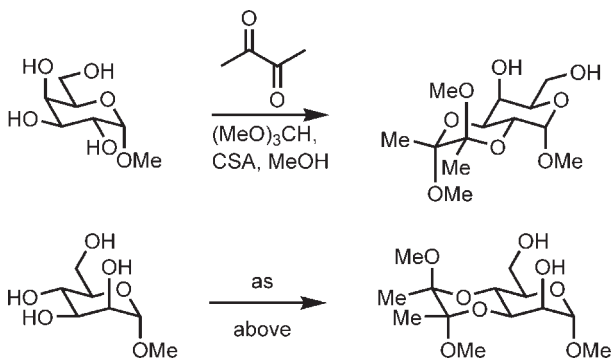
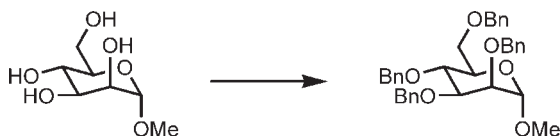


Figure 2.63

## 2.7 EXPERIMENTAL PROCEDURES

### Method 1 [87]

Preparation of methyl-2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-mannopyranoside.



BnBr, NaH, 0 °C  $\rightarrow$  rt, 17 h.

### Notes.

Alkylation under strongly basic conditions (anionic benzylation).

The use of a dipolar aprotic solvent (DMF) enhances the nucleophilicity of the alkoxide by solvating the metal counter-ion.

### Materials.

Benzyl bromide (9.19 ml, 77.0 mmol)	irritant, lachrymator
$\alpha$ -D-Mannopyranoside (3.00 g, 15.0 mmol)	assume toxic
<i>N,N</i> -dimethylformamide (anhydrous, 15 ml)	irritant, harmful
Sodium hydride (3.60 g, 90.0 mmol, 60% dispersion in oil)	irritant, harmful, reacts violently with water liberating hydrogen
Methanol (10 ml)	highly flammable, toxic



Diethyl ether (100 + 100 ml)	extremely flammable, harmful
Distilled water (150 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Hexane (chromatography solvent)	highly flammable, harmful
Ethyl acetate (chromatography solvent)	highly flammable, irritant

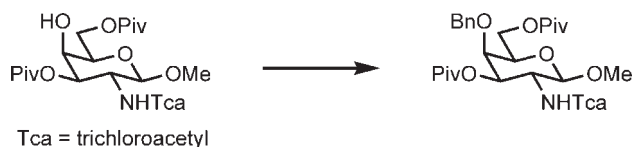
#### Equipment.

Round bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  
Source of inert gas  
Syringes (10 and 20 ml)  
Magnetic stirrer  
Ice bath  
Column chromatography equipment

*Procedure.* Add benzyl bromide (9.19 ml, 77.0 mmol) to a stirred solution of  $\alpha$ -D-mannopyranoside (3.00 g, 15.0 mmol) in anhydrous DMF (15 ml). Cool the reaction vessel to 0 °C and add sodium hydride (3.60 g, 90.0 mmol, 60% dispersion in oil) portion-wise, then allow the mixture to warm slowly to room temperature. After 17 h, the reaction should be complete by TLC analysis (3:1, hexane/ethyl acetate). Quench the reaction with methanol (10 ml), transfer the mixture to a separatory funnel, dilute with diethyl ether (100 ml) and wash with distilled water (150 ml). Back-extract the aqueous phase with diethyl ether (100 ml), then dry the combined organic layers ( $\text{MgSO}_4$ ) and remove solvents *in vacuo*. Purification of the crude residue by flash column chromatography (4:1, hexane/ethyl acetate) affords the product as a pale yellow syrup (7.30 g, 83%).  $[\alpha]_{\text{D}}^{22} = +29.2$  (*c* 1.59, chloroform);  $R_{\text{f}} = 0.60$  (3:1, hexane/ethyl acetate);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 3.35 (s,  $\text{OCH}_3$ , 3H), 3.74–3.83 (m, H-2, H-5 and  $2 \times$  H-6, 4H), 3.92 (dd,  $J = 3.0$  and 9.2 Hz, H-3, 1H), 4.02 (~t,  $J = 9.2$  and 9.2 Hz, H-4, 1H), 4.54 (d,  $J = 10.8$  Hz,  $\text{PhCH}_2$ , 1H), 4.58 (d,  $J = 12.1$  Hz,  $\text{PhCH}_2$ , 1H), 4.63 (s,  $\text{PhCH}_2$ , 2H), 4.70 (d,  $J = 12.1$  Hz,  $\text{PhCH}_2$ , 1H), 4.77 (s,  $\text{PhCH}_2$ , 2H), 4.81 (d,  $J = 1.7$  Hz, H-1, 1H), 4.92 (d,  $J = 10.8$  Hz,  $\text{PhCH}_2$ , 1H), 7.15–7.46 (m, ArH, 20H).

#### Method 2 [88]

Preparation of methyl-4-*O*-benzyl-2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido- $\beta$ -D-galactopyranoside.



NaH, BnBr, cat. Bu<sub>4</sub>NI, 0 °C → rt, 5 h.

#### Notes.

Alkylation under basic conditions (anionic benzylation).

The tetrabutylammonium iodide catalyst enhances the reactivity of the alkylating agent by generating benzyl iodide *in situ*.

The catalyst renders the use of a highly polar solvent such as DMF unnecessary, enabling a relatively non-polar solvent (THF) to be employed.

#### Materials.

Sodium hydride (1.00 g, 25.0 mmol, 60% dispersion in oil)	irritant, harmful, reacts violently with water liberating hydrogen
Methyl-2-deoxy-3,6-di- <i>O</i> -pivaloyl-2-trichloroacetamido-β-D-galactopyranoside (4.10 g, 8.10 mmol)	assume toxic
Tetrahydrofuran (anhydrous, 48 ml)	highly flammable, irritant
Tetrabutylammonium iodide (0.59 g, 1.6 mmol)	harmful, irritant
Benzyl bromide (2.40 ml, 20.3 mmol)	irritant, lachrymator
Glacial acetic acid (~ 1.50 ml)	flammable, corrosive
Ethyl acetate (150 ml + chromatography solvent)	highly flammable, irritant
Distilled water (150 ml)	no risk
Saturated solution of sodium hydrogen carbonate (150 ml)	no risk
Saturated solution of sodium chloride (100 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Petroleum ether (chromatography solvent)	highly flammable, harmful

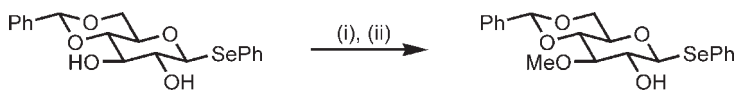
*Equipment.*

Round bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  
 Source of inert gas  
 Syringes (50 and 5 ml)  
 Magnetic stirrer  
 Ice bath  
 Column chromatography equipment

*Procedure.* Add sodium hydride (1.00 g, 25.0 mmol, 60% dispersion in oil) portion wise to a stirred solution of methyl-2-deoxy-3,6-di-*O*-pivaloyl-2-trichloro-acetamido- $\beta$ -D-galactopyranoside (4.10 g, 8.10 mmol) in anhydrous THF (48 ml) cooled to 0 °C. Stir the reaction mixture for 30 min at 0 °C then add tetrabutylammonium iodide (0.59 g, 1.6 mmol) followed by benzyl bromide (2.40 ml, 20.3 mmol) and stir at 0 °C for a further 1 h, then warm to room temperature and stir for 4 h. Carefully add glacial acetic acid (1.50 ml) and dilute the reaction mixture with ethyl acetate (150 ml). Wash the resulting solution successively with distilled water (150 ml), saturated aqueous NaHCO<sub>3</sub> solution (150 ml) and saturated aqueous NaCl solution (100 ml), dry the organic phase (MgSO<sub>4</sub>), filter off the drying agent and remove the solvent *in vacuo* to furnish the crude product. Purification by flash column chromatography (5:1, petrol/ethyl acetate) affords the product as a white foam (3.88 g, 80%).  $[\alpha]_D^{22} = -4.0$  (*c* 1.0, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.19 and 1.22 (2 × s, 2 × (CH<sub>3</sub>)<sub>3</sub>C, 18H), 3.49 (s, OCH<sub>3</sub>, 3H), 3.78 (m, H-5, 1H), 3.87 (dd, *J* = 3.0 and 9.0 Hz, H-4, 1H), 4.11 (dd, *J* = 6.2 and 11.0 Hz, H-6, 1H), 4.30 (m, H-2 and H-6, 2H) 4.57 (d, *J* = 8.4 Hz, H-1, 1H), 4.69 (m, PhCH<sub>2</sub>, 2H), 5.29 (dd, *J* = 3.0 and 11.1 Hz, H-3, 1H) 6.70 (d, *J* = 8.8 Hz, NH, 1H) 7.40 (m, ArH, 5H); MS *m/z* (CI), 615 (M + NH<sub>4</sub>), 566 (M – OMe).

*Method 3 [89]*

Preparation of phenyl-3-*O*-methyl-4,6-*O*-benzylidene-1-seleno- $\beta$ -D-glucopyranoside.



(i) NaH, rt, 1 h; (ii) CuCl<sub>2</sub>, MeI,  $\Delta$ , 24 h.

*Notes.*

Regioselective alkylation under basic conditions.

Chelation of the C-2 alkoxide with copper(II) chloride leaves the C-3 alkoxide free to react with the alkylating agent.

*Materials.*

Sodium hydride (0.04 g, 1.00 mmol, 60% dispersion in oil)	irritant, harmful, reacts violently with water liberating hydrogen
Phenyl-4,6- <i>O</i> -benzylidene-1-seleno- $\beta$ -D-glucopyranoside (0.20 g, 0.50 mmol)	assume toxic
Tetrahydrofuran (anhydrous, 10 ml)	highly flammable, irritant
Copper(II) chloride (67.0 mg, 0.50 mmol)	harmful, irritant
Methyl iodide (0.16 ml, 2.50 mmol)	harmful, irritant, toxic
Distilled water (3 ml)	no risk
Ammonia solution (50% aqueous, 3 ml)	corrosive, irritant
Ethyl acetate (5 $\times$ 5 ml)	highly flammable, irritant
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Dichloromethane (chromatography solvent)	toxic, irritant, possible irreversible effects
Acetone (chromatography solvent)	highly flammable
Triethylamine	highly flammable, corrosive, harmful

*Equipment.*

Drying pistol  
Two-necked round bottomed flask (25 ml) with rubber septum and magnetic stirrer bar  
Reflux condenser  
Source of inert gas  
Heater stirrer with thermostat thermometer  
Oil bath  
Syringes (10 ml and 200  $\mu$ l)  
Column chromatography equipment

*Procedure.* Before the protection reaction is commenced, the copper(II) chloride must be dried under reduced pressure (high vacuum,  $\sim$ 0.1 mm Hg) at 80  $^{\circ}$ C for 24 h.

Add sodium hydride (0.04 g, 1.00 mmol, 60% dispersion in oil) portion-wise to a stirred solution of phenyl-4,6-*O*-benzylidene-1-seleno- $\beta$ -D-glucopyranoside (0.20 g, 0.50 mmol) in anhydrous THF (10 ml). Stir the reaction mixture for 1 h at room temperature; during this time a thick white slurry will form and hydrogen will be evolved. Add anhydrous copper(II) chloride (67.0 mg, 0.50 mmol) whereupon the white slurry will immediately dissolve to form a dark green solution. Add methyl iodide (0.16 ml, 2.50 mmol) and heat the reaction mixture at reflux for 24 h. Allow the reaction vessel to cool to room temperature and pour into a separatory funnel containing distilled water (3 ml) and 50% aqueous ammonia solution (3 ml) to afford a dark blue solution. Separate the organic layer and extract the aqueous phase with ethyl acetate (5  $\times$  5 ml), dry the combined organic layers ( $\text{MgSO}_4$ ) and remove solvents *in vacuo* to furnish the crude product as a pale yellow oil. Purification by flash column chromatography (10:1, dichloromethane/acetone + 1% triethylamine) affords phenyl-3-*O*-methyl-4,6-*O*-benzylidene-1-seleno- $\beta$ -D-glucopyranoside as a colourless oil (0.15 g, 75%).  $[\alpha]_{\text{D}}^{20} = -24.0$  ( $c$  1.01, chloroform); IR (liquid film)/ $\text{cm}^{-1}$  3334 br (O–H), 2980 s (C–H), 2854 s (C–H), 1445 s (C–H), 1372 vs (C–H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.68 (d,  $J = 2.0$  Hz, OH, 1H), 3.40 (s,  $\text{OCH}_3$ , 3H), 3.47–3.49 (m, H-2 and H-5, 2H), 3.57–3.59 (m, H-4, 1H), 3.66 (t,  $J = 9.8$  Hz, H-6, 1H), 3.72–3.78 (m, H-3, 1H), 4.19–4.23 (dd,  $J = 5.0$  and 9.8 Hz, H-6, 1H), 4.94 (d,  $J = 2.5$  Hz, H-1, 1H), 5.74 (s,  $\text{PhCH}$ , 1H), 7.28–7.44 (m, Ar-H, 10H); MS  $m/z$  (CI) 423 (M + H, 21%), 265 (65), 205 (57), 105 (100).

#### Method 4 [90]

Preparation of 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl fluoride.



$\text{BnBr}$ ,  $\text{Ag}_2\text{O}$ , rt, 4 h.

#### Notes.

Alkylation under essentially neutral conditions.

The silver(I) oxide aids dissociation of bromide, enhancing the reactivity of the alkylating agent.

#### Materials.

Solution of sodium hydroxide (10 g in 100 ml water)	irritant, harmful
Solution of silver nitrate (39 g in 250 ml water)	corrosive
Distilled water (4 $\times$ 100 ml)	no risk
$\alpha$ -D-Glucopyranosyl fluoride (2.60 g, 14.3 mmol)	assume toxic
<i>N,N</i> -dimethylformamide (anhydrous, 50 ml)	irritant, harmful

Benzyl bromide (13.0 ml, 109 mmol)	irritant, lachrymator
Silver(I) oxide (26.5 g, 114 mmol)	irritant
Methanol (10 ml)	highly flammable, toxic
Celite <sup>®</sup>	harmful by inhalation, possible irreversible effects
Hexane (chromatography solvent)	highly flammable, harmful
Diethyl ether (chromatography solvent)	extremely flammable, harmful

*Equipment.*

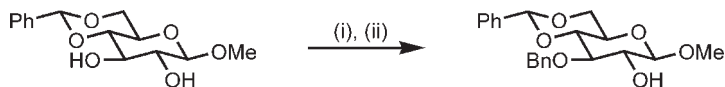
Round bottomed flask (250 ml) with rubber septum and magnetic stirrer bar  
Source of inert gas  
Syringes (50 and 20 ml)  
Magnetic stirrer  
Cold water bath  
Column chromatography equipment

*Procedure.* The silver(I) oxide should be prepared directly before use; add a solution of sodium hydroxide (10 g, 0.25 mol) in water (100 ml) to a stirring solution of silver nitrate (39 g, 0.23 mol) in water (250 ml). Filter off the precipitated silver(I) oxide and wash thoroughly with distilled water ( $2 \times 100$  ml), then dry under reduced pressure (high vacuum,  $\sim 0.1$  mm Hg) at  $80^\circ\text{C}$  for 24 h.

Dissolve  $\alpha$ -D-glucopyranosyl fluoride (2.60 g, 14.3 mmol) in anhydrous DMF (50 ml), then add benzyl bromide (13.0 ml, 109 mmol) and place the reaction vessel in a cooling water bath. Next add the freshly prepared silver(I) oxide (26.5 g, 114 mmol) and stir the reaction at room temperature for 4 h. Quench the reaction by adding methanol (10 ml) and stirring for 30 min to destroy excess alkylating agent. Filter through a pad of Celite<sup>®</sup> and concentrate the filtrate *in vacuo* to afford the crude product. Purification by flash column chromatography (5:1, hexane/diethyl ether) furnishes colourless crystals (6.50 g, 84%). Mp  $70.5^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{20} = +4.2$  (*c* 0.87, chloroform);  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ ) 3.32 (m,  $J = 2.6, 9.8$  and  $25.8$  Hz, H-2, 1H), 3.55 (dd,  $J = 1.8$  and  $11.0$  Hz, H-6, 1H), 3.83 (dd,  $J = 3.1$  and  $11.0$  Hz, H-6, 1H), 3.90 (dd,  $J = 9.2$  and  $10.0$  Hz, H-4, 1H), 4.08 (m, H-5, 1H), 4.14 (dd,  $J = 9.2$  and  $9.8$  Hz, H-3, 1H), 4.22–4.92 (m,  $4 \times \text{PhCH}_2$ , 8H), 5.61 (dd,  $J = 2.6$  and  $53.6$  Hz, H-1, 1H), 7.02–7.38 (m, ArH, 20H).

*Method 5 [91]*

Preparation of methyl-3-*O*-benzyl-4,6-*O*-benzylidene- $\beta$ -D-glucopyranoside.



(i)  $\text{Bu}_2\text{SnO}$ ,  $\Delta$ , 16 h; (ii)  $\text{BnBr}$ ,  $\text{Bu}_4\text{NI}$ ,  $\Delta$ , 16 h.

*Notes.*

Regioselective alkylation under essentially neutral conditions.

The reaction proceeds via the formation of a dibutylstannylene acetal; in this example alkylation occurs at the C-3 hydroxyl.

Dibutyltin dimethoxide can also be employed for the selective alkylation and acylation of diols [91b,c].

*Materials.*

Methyl-4,6- <i>O</i> -benzylidene- $\beta$ -D-glucopyranoside (8.00 g, 28.3 mmol)	assume toxic
Dibutyltin oxide (8.41 g, 33.8 mmol)	toxic, avoid contact with eyes
Benzene (400 ml)	highly flammable, toxic, carcinogen
Benzyl bromide (3.70 ml, 31.1 mmol)	irritant, lachrymator
Tetrabutylammonium iodide (11.9 g, 32.1 mmol)	harmful, irritant
Methanol	highly flammable, toxic

*Equipment.*

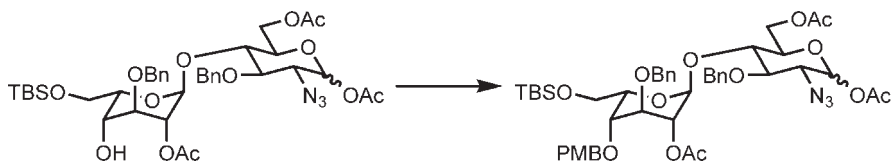
Round bottomed flask (500 ml) with magnetic stirrer bar  
 Dean and Stark trap  
 Reflux condenser  
 Heater stirrer with thermostat thermometer  
 Oil bath  
 Source of inert gas  
 Syringe (5 ml)

*Procedure.* Place methyl-4,6-*O*-benzylidene- $\beta$ -D-glucopyranoside (8.00 g, 28.3 mmol), dibutyltin oxide (8.41 g, 33.8 mmol) and benzene (400 ml) in a round-bottomed flask equipped with a Dean and Stark trap and a reflux condenser. Stir the solution at reflux for 16 h with azeotropic removal of water. Allow

the solution to cool to room temperature, then concentrate *in vacuo* to approximately 200 ml. Add benzyl bromide (3.70 ml, 31.1 mmol) and tetrabutylammonium iodide (11.9 g, 32.1 mmol) and heat the reaction mixture at reflux for a further 16 h. Cool the solution to room temperature and remove the solvents *in vacuo* to afford the crude product as a yellow solid. Recrystallise the crude product from methanol to furnish methyl-3-*O*-benzyl-4,6-*O*-benzylidene- $\beta$ -D-glucopyranoside as a white crystalline solid (8.40 g, 80%). Mp 182–183 °C;  $[\alpha]_D = -51.0$  (*c* 0.86, chloroform);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.44 (d,  $J = 2.2$  Hz, OH, 1H), 3.57 (s,  $\text{OCH}_3$ , 3H), 3.80 (~t,  $J = 10.2$  Hz, H-4, 1H), 4.33 (d,  $J = 7.6$  Hz, H-1, 1H), 4.36 (dd,  $J = 5.0$  and 10.5 Hz, H-6, 1H), 4.79 (d,  $\text{PhCH}_2$ , 1H), 4.97 (d,  $\text{PhCH}_2$ , 1H), 5.58 (s,  $\text{PhCH}$ , 1H), 7.25–7.48 (m, ArH, 10H); MS  $m/z$  (ESI), 373 ( $\text{M} + \text{Na}$ ).

#### Method 6 [92]

Preparation of di-1,6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-benzyl-4-*O*-*para*-methoxybenzyl-6-*O*-*tert*-butyldimethylsilyl- $\alpha$ -L-idopyranosyl)-D-glucopyranose.



$\text{PMBO-C(=NH)CCl}_3$ , cat. TfOH, rt, 2 h.

#### Notes.

Alkylation under mildly acidic conditions.

Protonation of the alkyl trichloroacetimidate (at nitrogen) activates the electrophile towards nucleophilic attack by the hydroxyl group of the sugar to generate the alkyl ether and liberate trichloroacetamide.

Acid labile groups (acetals) can survive these mildly acidic reaction conditions.

#### Materials.

Sodium hydride (0.50 g, 21.0 mmol)

irritant, harmful,  
reacts violently  
with water  
liberating hydrogen

Diethyl ether (anhydrous, 20 + 30 ml)

extremely  
flammable, harmful

*para*-Methoxybenzyl alcohol (29.0 g, 210 mmol)

harmful, irritant



Trichloroacetonitrile (20.0 ml, 200 mmol)	toxic, lachrymator
Pentane (20 + 2 × 20 ml)	extremely flammable, harmful
Methanol (anhydrous, ~0.8 ml)	highly flammable, toxic
Di-1,6- <i>O</i> -acetyl-2-azido-3- <i>O</i> -benzyl-2-deoxy-4- <i>O</i> -(2- <i>O</i> -acetyl-3- <i>O</i> -benzyl-6- <i>O</i> - <i>tert</i> -butyldimethylsilyl- $\alpha$ -L-idopyranosyl)-D-glucopyranose (720 mg, 0.91 mmol)	assume toxic
Diethyl ether (anhydrous, 15 ml)	extremely flammable, harmful
Dichloromethane (anhydrous, 20 ml and chromatography solvent)	toxic, irritant, possible irreversible effects
<i>para</i> -Methoxybenzyl trichloroacetimidate (513 mg, 1.82 mmol)	toxic, irritant
Solution of triflic acid in diethyl ether (0.01 M, 0.1 ml)	corrosive, harmful
Triethylamine (0.14 ml, 1.00 mmol)	highly flammable, corrosive, harmful
Ethyl acetate (chromatography solvent)	highly flammable, irritant

### *Equipment.*

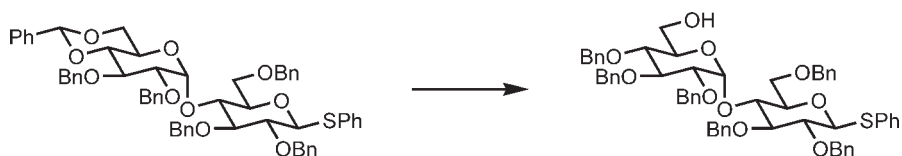
Round bottomed flask (250 ml) with rubber septum and magnetic stirrer bar  
 Round bottomed flask (100 ml) with rubber septum  
 Source of inert gas  
 Syringes (50 and 20 ml)  
 Cannula  
 Magnetic stirrer  
 Ice-salt bath  
 Round bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  
 Source of inert gas  
 Syringes (20, 5 ml, 100 and 200  $\mu$ l)  
 Magnetic stirrer  
 Column chromatography equipment

*Procedure.* The alkyl trichloroacetimidate can be prepared as follows. To a stirred suspension of sodium hydride (0.50 g, 21.0 mmol) in anhydrous diethyl ether (20 ml) add dropwise via cannula a solution of *para*-methoxybenzyl alcohol (29.0 g, 210 mmol) in diethyl ether (30 ml). After the solids have dissolved, cool the reaction vessel in an ice–salt bath and add trichloroacetonitrile (20.0 ml, 200 mmol) over a period of 15 min, then allow the reaction mixture to warm to room temperature. After 1 h concentrate the solution *in vacuo* to afford a syrup, then add pentane (20 ml) containing anhydrous methanol (0.8 ml) and stir vigorously. Filter off the precipitate and wash with pentane (2 × 20 ml); concentrate the filtrate and washings *in vacuo* to yield the alkyl trichloroacetimidate.

To a stirred solution of the disaccharide (720 mg, 0.91 mmol) in anhydrous diethyl ether/dichloromethane (3:1, 20 ml) add *para*-methoxybenzyl trichloroacetimidate (513 mg, 1.82 mmol) followed by a solution of triflic acid in diethyl ether (0.01 M, 0.1 ml). Stir the reaction at room temperature for 2 h, then neutralise with triethylamine (0.14 ml, 1.00 mmol) and concentrate the solution *in vacuo*. Purification of the crude product by column chromatography on Sephadex LH20 (1:1, dichloromethane/ethyl acetate), affords the ether (745 mg, 90%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , A:  $\alpha$  anomer, B:  $\beta$  anomer) 0.02 and 0.09 ( $2 \times \text{s}$ ,  $2 \times \text{Si}(\text{CH}_3)_2$  A and B, 12H), 0.89 (s,  $2 \times (\text{CH}_3)_3\text{CSi}$  A and B, 18H), 2.03, 2.06, 2.14 and 2.15 ( $4 \times \text{s}$ ,  $6 \times \text{OAc}$  A and B, 18H), 3.42–3.60 (m, H-2A, H-2B, H-4'A, H-4'B and H-5B, 5H), 3.65–3.90 (m,  $2 \times \text{OCH}_3$  A and B, H-3A, H-3B, H-3'A, H-3'B, H-4A, H-4B, H-5A,  $2 \times \text{H-6'A}$ ,  $2 \times \text{H-6'B}$ , 17H), 4.09–4.15 (m, H-5'A and H-5'B, 2H), 4.20 (dd,  $J = 4.0$  and 12.3 Hz, H-6B, 1H), 4.22 (dd,  $J = < 1.0$  and 12.1 Hz, H-6A, 1H), 4.31 (dd,  $J = < 1.0$  and 12.1 Hz, H-6A, 1H), 4.40 (dd,  $J = 2.2$  and 12.3 Hz, H-6B, 1H), 4.40–5.05 (m,  $6 \times \text{PhCH}_2$  A and B, H-1'A, H-1'B, H-2'A, H-2'B, 16H), 5.41 (d,  $J = 8.4$  Hz, H-1B, 1H), 6.17 (d,  $J = 3.6$  Hz, H-1A, 1H), 6.78–7.13 (m, ArH, 4H), 7.17–7.33 (m, ArH, 24H).

### Method 7 [93]

Preparation of phenyl-2,3,6,2',3',4'-hexa-*O*-benzyl-1-thio- $\beta$ -D-maltoside.



$\text{LiAlH}_4$ ,  $\text{AlCl}_3$ , 50 °C, 1 h.

### Notes.

Regioselective reductive ring opening of a benzylidene acetal.  
Steric interactions determine the observed selectivity.

*Materials.*

Lithium aluminium hydride (2.00 g, 52.7 mmol)	reacts violently with water liberating hydrogen assume toxic
Phenyl-2,3,6-tri- <i>O</i> -benzyl-4- <i>O</i> -(2,3-di- <i>O</i> -benzyl-4,6-benzylidene- $\alpha$ -D-glucopyranosyl)-1-thio- $\beta$ -D-glucopyranoside (10.4 g, 10.7 mmol)	
Dichloromethane (anhydrous, 100 + 4 $\times$ 100 ml)	toxic, irritant, possible irreversible effects
Diethyl ether (anhydrous, 40 + 60 ml)	extremely flammable, harmful
Aluminium chloride (4.30 g, 32.2 mmol)	corrosive
Ethyl acetate (100 ml and chromatography solvent)	highly flammable, irritant
Distilled water (100 ml)	no risk
Hydrochloric acid (1.0 M, 100 ml)	corrosive, toxic
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Cyclohexane (chromatography solvent)	highly flammable, irritant, harmful

*Equipment.*

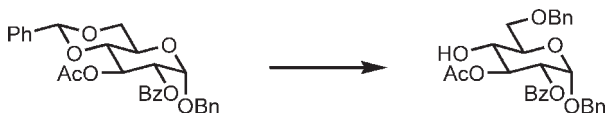
Two-necked round bottomed flask (250 ml) fitted with rubber septum, 100 ml dropping funnel and magnetic stirrer bar  
 Reflux condenser  
 Round bottomed flask (100 ml) with rubber septum  
 Source of inert gas  
 Wide-bore cannula  
 Syringes (2  $\times$  50 ml)  
 Heater stirrer with thermostat thermometer  
 Oil bath  
 Ice bath  
 Column chromatography equipment

*Procedure.* Carefully add lithium aluminium hydride (2.00 g, 52.7 mmol) to a stirred solution of phenyl-2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-benzylidene- $\alpha$ -D-glucopyranosyl)-1-thio- $\beta$ -D-glucopyranoside (10.4 g, 10.7 mmol) in

anhydrous dichloromethane (100 ml) and anhydrous diethyl ether (40 ml). Add a stirring suspension of aluminium chloride (4.30 g, 32.2 mmol) in anhydrous diethyl ether (60 ml) via wide-bore cannula over 15 min and heat the resulting mixture to 50 °C. After 1 h cool the mixture to 0 °C and carefully add ethyl acetate (100 ml) dropwise (dropping funnel), followed by distilled water (100 ml) and hydrochloric acid (1.0 M, 100 ml). Transfer the mixture to a separatory funnel, separate the organic layer and extract the aqueous phase with dichloromethane (4 × 100 ml). Dry the combined organic extracts (MgSO<sub>4</sub>) and concentrate *in vacuo* to furnish the crude product. Purification by flash column chromatography with gradient elution (3:17 → 1:4, ethyl acetate/cyclohexane) affords phenyl-2,3,6,2',3',4'-hexa-*O*-benzyl-1-thio-β-D-maltoside as a colourless oil (9.76 g, 94%). [ $\alpha$ ]<sub>D</sub><sup>21</sup> = +24.5 (*c* 0.8, chloroform); *R*<sub>f</sub> = 0.40 (2:5, ethyl acetate/cyclohexane); IR (KBr disc)/cm<sup>-1</sup> 3481 (O–H), 1079 (C–O); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.75 (dd, *J* = 4.3 and 8.5 Hz, OH, 1H), 3.48 (dd, *J* = 3.6 and 9.5 Hz, H-2', 1H), 3.56 (t, *J* = 9.5 Hz, H-4', 1H), 3.63 (t, *J* = 9.2 Hz, H-2, 1H), 3.59–3.66 (m, H-5 and H-6', 2H), 3.71 (ddd, *J* = 3.0, 4.3 and 11.9 Hz, H-6', 1H), 3.76 (dt, *J* = 3.0 and 9.5 Hz, H-5', 1H), 3.85 (dd, *J* = 2.1 and 11.2 Hz, H-6, 1H), 3.87 (t, *J* = 9.2 Hz, H-4, 1H), 3.92 (dd, *J* = 3.8 and 11.2 Hz, H-6, 1H), 4.00 (t, *J* = 9.5 Hz, H-3', 1H), 4.16 (t, *J* = 9.2 Hz, H-3, 1H), 4.56 (d, *J* = 12.0 Hz, PhCH, 1H), 4.61–4.71 (m, 5 × PhCH, 5H), 4.75 (d, *J* = 9.2 Hz, H-1, 1H), 4.85 (d, *J* = 10.9 Hz, PhCH, 1H), 4.88–4.95 (m, 4 × PhCH, 4H), 4.97 (d, *J* = 11.7 Hz, PhCH, 1H), 5.65 (d, *J* = 3.6 Hz, H-1', 1H), 7.23–7.66 (m, ArH, 35H); MS *m/z* (CI), 992 (M + NH<sub>4</sub>, 100%).

#### Method 8 [94]

Preparation of benzyl-3-*O*-acetyl-2-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside.



NaBH<sub>3</sub>CN, HCl in Et<sub>2</sub>O, rt.

#### Notes.

Regioselective reductive ring opening of a benzylidene acetal.

Acetal oxygen basicity determines the observed selectivity.

#### Materials.

Solution of hydrogen chloride in diethyl ether (1.0 M, few ml) extremely flammable, harmful, causes severe burns

Benzyl-3- <i>O</i> -acetyl-2- <i>O</i> -benzoyl-4,6- <i>O</i> -benzylidene- $\alpha$ -D-glucopyranoside (0.93 g, 1.84 mmol)	assume toxic
Sodium cyanoborohydride (1.45 g, 23.1 mmol)	toxic, highly flammable, corrosive
3 Å molecular sieves ( $\sim$ 0.5 g)	irritant
Tetrahydrofuran (15 ml)	highly flammable, irritant
Dichloromethane (100 ml)	toxic, irritant, possible irreversible effects
Celite <sup>®</sup>	harmful by inhalation, possible irreversible effects
Saturated solution of sodium hydrogen carbonate (2 $\times$ 150 ml)	no risk
Distilled water (100 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation

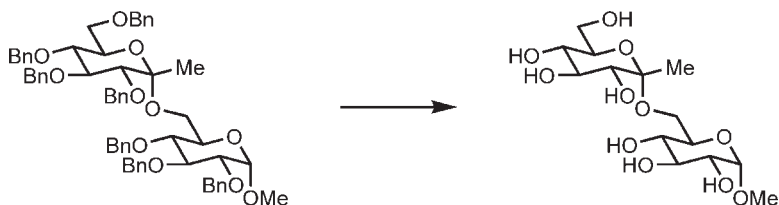
### Equipment.

Round bottomed flask (50 ml) with rubber septum and magnetic stirrer bar  
 Syringe (20 ml)  
 Magnetic stirrer  
 Column chromatography equipment

**Procedure.** Add dropwise a solution of hydrogen chloride in diethyl ether (1.0 M) to a stirred suspension of benzyl-3-*O*-acetyl-2-*O*-benzoyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside (0.93 g, 1.84 mmol), sodium cyanoborohydride (1.45 g, 23.1 mmol) and 3 Å molecular sieves ( $\sim$ 0.5 g) in THF (15 ml). Once the evolution of hydrogen has ceased dilute the reaction mixture with dichloromethane (100 ml) and filter the mixture through a Celite<sup>®</sup> pad. Wash the filtrate with saturated aqueous NaHCO<sub>3</sub> solution (2  $\times$  150 ml) and distilled water (100 ml); dry the combined organic layers (MgSO<sub>4</sub>) and remove solvents *in vacuo* to furnish the crude product. Purification by flash column chromatography affords the product in 82% yield (0.77 g).  $[\alpha]_D = +118.1$  (c 1.1, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.02 (s, OAc, 3H), 2.88 (d, *J* = 3.8 Hz, OH, 1H), 3.70 (dd, *J* = 4.0, 10.4 Hz, H-6, 1H), 3.78 (dd, *J* = 4.2, 10.4 Hz, H-6, 1H), 3.84–3.88 (m, H-4, 1H), 3.91–3.97 (m, H-5, 1H), 4.52 (d, *J* = 12.4 Hz, PhCH<sub>2</sub>, 1H), 4.57 (d, *J* = 12.0 Hz, PhCH<sub>2</sub>, 1H), 4.63 (d, *J* = 12.0 Hz, PhCH<sub>2</sub>, 1H), 4.73 (d, *J* = 12.4 Hz, PhCH<sub>2</sub>, 1H), 5.04 (dd, *J* = 3.7, 10.2 Hz, H-2, 1H), 5.23 (d, *J* = 3.7 Hz, H-1, 1H), 5.59 (dd, *J* = 9.1, 10.2 Hz, H-3, 1H).

*Method 9 [95]*

Preparation of methyl-*O*-(1'-*C*-methyl- $\alpha$ -D-glucopyranosyl)-(1'  $\rightarrow$  6)- $\alpha$ -D-glucoside.



$\text{H}_2$ , cat.  $\text{Pd}(\text{OH})_2/\text{C}$ , rt, 12 h.

*Notes.*

Palladium on carbon and palladium(II) acetate are also commonly used to effect deprotection.

*Materials.*

Pearlman's catalyst (palladium(II) hydroxide on carbon, 20% by weight, 200 mg)	harmful, irritant
1'- <i>C</i> -methyl- $\alpha$ -D-disaccharide (1.00 g, 1.00 mmol)	assume toxic
Methanol (20 + 3 $\times$ 5 ml + chromatography solvent)	highly flammable, toxic
Hydrogen	highly flammable, forms explosive mixtures with air
Celite <sup>®</sup>	harmful by inhalation, possible irreversible effects
Dichloromethane (chromatography solvent)	toxic, irritant, possible irreversible effects

*Equipment.*

Round bottomed flask (100 ml) with magnetic stirrer bar  
Quickfit three-way tap

Magnetic stirrer

Source of argon

Column chromatography equipment

**Procedure.** Add Pearlman's catalyst ( $\text{Pd}(\text{OH})_2/\text{C}$ , 20 wt%, 200 mg) to a 100 ml round-bottomed flask containing a solution of the 1'-*C*-methyl- $\alpha$ -D-disaccharide (1.00 g, 1.00 mmol) in methanol (20 ml). Stopper the flask with a Quickfit three-way tap. Attach a balloon filled with argon to one inlet and connect the other inlet to a water aspirator. Evacuate the flask and then purge with argon, repeat this twice, so that the reaction vessel is free of oxygen. Next swap the argon balloon for a balloon containing hydrogen, evacuate the flask again and purge with hydrogen, repeat this three times. Disconnect the aspirator inlet and stir the reaction mixture vigorously at room temperature under a positive pressure of hydrogen for 12 h. Once the reaction is complete purge the flask with argon. Remove the catalyst by filtration through a pad of Celite<sup>®</sup>, wash with methanol ( $3 \times 5$  ml) and concentrate the filtrate *in vacuo*. Submit the residue to flash column chromatography (2:1, dichloromethane/methanol) to furnish the debenzylated product as a colourless glassy solid (369 mg, 100%).  $[\alpha]_{\text{D}}^{23} = +126.19$  (*c* 1.57, methanol);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) 1.34 (s,  $\text{CH}_3$ , 3H), 3.05 (d,  $J = 9.5$  Hz, H-2', 1H), 3.22–3.23 (m,  $2 \times$  H-6, 2H), 3.31–3.33 (m, H-6', 1H), 3.34 (s,  $\text{OCH}_3$ , 3H), 3.50–3.71 (m, H-2, H-3, H-3', H-4, H-4', H-5, H-5' and H-6', 8H), 4.59 (d,  $J = 4.0$  Hz, H-1, 1H).

### Method 10 [96]

Preparation of 4-*O*-allyl-1,2,3,6-tetra-*O*-acetyl- $\alpha$ - $\beta$ -D-glucopyranose.



$\text{AllylOCO}_2\text{Et}$ , DPPB, cat.  $\text{Pd}_2\text{dba}_3$ , 60 °C, 2 h.

### Notes.

A convenient one-pot procedure for allylation under neutral conditions.

### Materials.

1,2,3,6-Tetra-*O*-acetyl- $\alpha$ - $\beta$ -D-glucopyranose (1.20 g, 3.45 mmol)

assume toxic

Tetrahydrofuran (anhydrous, 30 + 15 ml)

highly flammable,  
irritant

Tris(dibenzylideneacetone)dipalladium(0) (55.0 mg, 60.0 $\mu\text{mol}$ )	harmful, irritant
1,4-Bis(diphenylphosphino)butane (160 mg, 0.38 mmol)	irritant
Allyl ethyl carbonate (2.00 g, 15.4 mmol)	irritant
Dichloromethane (chromatography solvent)	toxic, irritant, possible irreversible effects
Methanol (chromatography solvent)	highly flammable, toxic

### Equipment.

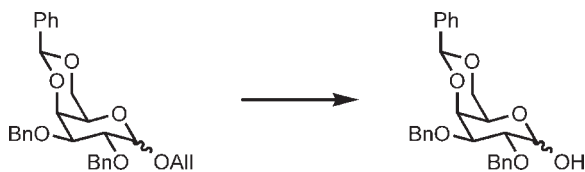
Round bottomed flask (25 ml) with rubber septum  
 Wide-bore cannula  
 Two-necked round bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  
 Air condenser  
 Source of inert gas  
 Syringe (50 ml)  
 Heater stirrer with thermostat thermometer  
 Oil bath  
 Column chromatography equipment

**Procedure.** To a stirred solution of 1,2,3,6-tetra-*O*-acetyl- $\alpha$ - $\beta$ -D-glucopyranose (1.20 g, 3.45 mmol) in anhydrous THF (30 ml) add via cannula a solution of tris (dibenzylideneacetone)dipalladium(0) (55.0 mg, 60.0  $\mu\text{mol}$ ) and 1,4-bis(diphenylphosphino)butane (160 mg, 0.38 mmol) in anhydrous THF (15 ml). Next add allyl ethyl carbonate (2.00 g, 15.4 mmol) and heat the solution to 60 °C. After 2 h cool the reaction mixture to room temperature, remove solvents *in vacuo* and purify directly by flash column chromatography (49:1, dichloromethane/methanol) to give 4-*O*-allyl-1,2,3,6-tetra-*O*-acetyl- $\alpha$ - $\beta$ -D-glucopyranose as an oil (1.20 g, 90%), ( $\alpha$ : $\beta$ , 3:1)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\alpha$ -anomer: 1.97 (s, OAc, 3H), 2.05 (s, OAc, 3H), 2.07 (s, OAc, 3H), 2.12 (s, OAc, 3H), 3.55 (~t,  $J$  = 10.0 Hz, H-4, 1H), 3.90–4.00 (m, H-5, 1H), 4.05 (d,  $J$  = 5.5 Hz,  $\text{OCH}_2\text{—CH=CH}_2$ , 2H), 4.20–4.35 (m,  $2 \times$  H-6, 2H), 5.00 (dd,  $J$  = 3.5, 10.0 Hz, H-2, 1H), 5.10–5.30 (m,  $\text{—CH=CH}_2$ , 2H), 5.45 (~t,  $J$  = 10.0 Hz, H-3, 1H), 5.70–5.85 (m,  $\text{—CH=CH}_2$ , 1H), 6.20 (d,  $J$  = 3.5 Hz, H-1, 1H).  $\beta$ -anomer: 1.98 (s, OAc, 3H), 2.03 (s, OAc, 3H), 2.06 ( $2 \times$  s,  $2 \times$  OAc, 6H), 3.65 (dd,  $J$  = 9.1, 10.0 Hz, H-4, 1H), 3.65–3.70 (m, H-5, 1H), 4.05 (d,  $J$  = 5.1 Hz,  $\text{OCH}_2\text{—CH=CH}_2$ , 2H), 4.20–4.35 (m,  $2 \times$  H-6, 2H), 5.00 (dd,  $J$  = 8.0, 10.4 Hz, H-2, 1H), 5.10–5.20 (m,  $\text{—CH=CH}_2$  and H-3, 3H), 5.65 (d,  $J$  = 8.0 Hz, H-1, 1H), 5.70–5.80 (m,  $\text{CH=CH}_2$ , 1H).



*Method 11 [97]*

Preparation of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-galactopyranose.



Cat. PdCl<sub>2</sub>, NaOAc, aqueous AcOH, rt.

*Notes.*

Transition-metal catalysed deallylation.

A convenient one-pot isomerisation/hydrolysis protocol.

*Materials.*

Palladium(II) chloride (33.4 mg, 0.19 mmol)	harmful, irritant
Allyl-2,3-di- <i>O</i> -benzyl-4,6- <i>O</i> -benzylidene-D-galactopyranoside (1.50 g, 3.10 mmol)	assume toxic
Solution of acetic acid (95% aqueous, 67 ml)	flammable, corrosive
Sodium acetate (2.10 g, 25.6 mmol)	irritant
Celite <sup>®</sup>	harmful by inhalation, possible irreversible effects
Dichloromethane (100 ml)	toxic, irritant, possible irreversible effects
Distilled water (50 ml)	no risk
Saturated solution of sodium hydrogen carbonate (2 × 30 ml)	no risk
Saturated solution of sodium chloride (50 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Petroleum ether (chromatography solvent)	highly flammable, harmful
Ethyl acetate (chromatography solvent)	highly flammable, irritant

*Equipment.*

Round bottomed flask (100 ml) with magnetic stirrer bar

Magnetic stirrer

Column chromatography equipment

*Procedure.* Add palladium(II) chloride (33.4 mg, 0.19 mmol) to a stirred solution of allyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene- $\beta$ -D-galactopyranoside (1.50 g, 3.10 mmol) in 95% aqueous acetic acid (67 ml) containing sodium acetate (2.10 g, 25.6 mmol). Stir the reaction mixture at room temperature until TLC analysis indicates that the starting material has been consumed. Once the reaction has gone to completion filter the mixture through a pad of Celite<sup>®</sup>, dilute the filtrate with dichloromethane (100 ml) and transfer to a separatory funnel. Wash successively with distilled water (50 ml), saturated aqueous NaHCO<sub>3</sub> solution (2  $\times$  30 ml), and saturated aqueous NaCl solution (50 ml). Dry the organic phase (MgSO<sub>4</sub>) and concentrate *in vacuo* to afford a dark yellow syrup. Purification by flash column chromatography (2:1, petrol/ethyl acetate) provides 2,3-di-*O*-benzyl-4,6-*O*-benzylidene- $\beta$ -D-galactopyranose as a solid (1.02 g, 74%,  $\alpha$ : $\beta$  mixture). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 5.37 (d, H-1 $\alpha$ , 1H), 5.49 and 5.50 (2  $\times$  s, PhCH  $\alpha$  and  $\beta$ , 2H), 7.26–7.37 (m, ArH  $\alpha$  and  $\beta$ , 30H). The allyl group protons which are present in the <sup>1</sup>H NMR spectrum of the starting material at  $\sim$ 3.98 (OCH<sub>2</sub>–CH=CH<sub>2</sub>, 2H),  $\sim$ 5.17 (–CH=CH<sub>2</sub>, 2H) and  $\sim$ 5.88 (–CH=CH<sub>2</sub>, 1H) will have disappeared from the spectrum of deprotected pyranose.

*Method 12 [98]*Preparation of ethyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio- $\alpha$ -D-galactopyranoside.TBDPSCl, imidazole, 0 °C  $\rightarrow$  rt, 24 h.*Notes.*

Selective silylation of the most accessible (1°) hydroxyl group.

Sterically hindered hydroxyl groups often require the silyl triflate rather than the silyl chloride; in such reactions scrupulously anhydrous conditions are essential.

*Materials.*Ethyl-1-thio- $\alpha$ -D-galactopyranoside (1.43 g, 6.38 mmol)

assume toxic

Imidazole (871 mg, 12.8 mmol)

harmful, corrosive

<i>N,N</i> -dimethylformamide (anhydrous, 60 ml)	irritant, harmful
<i>tert</i> -Butyldiphenylsilyl chloride (1.83 ml, 7.04 mmol)	corrosive
Ethyl acetate (100 ml + 3 × 50 ml + chromatography solvent)	highly flammable, irritant
Distilled water (150 ml)	no risk
Saturated solution of sodium chloride (200 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation

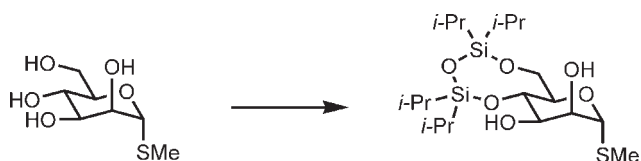
### Equipment.

Round bottomed flask (250 ml) with rubber septum and magnetic stirrer bar  
 Source of inert gas  
 Syringe (50 ml)  
 Magnetic stirrer  
 Ice bath  
 Column chromatography equipment

**Procedure.** Dissolve ethyl-1-thio- $\alpha$ -D-galactopyranoside (1.43 g, 6.38 mmol) and imidazole (871 mg, 12.8 mmol) in anhydrous DMF (60 ml) and cool the stirred solution to 0 °C. Add *tert*-butyldiphenylsilyl chloride (1.83 ml, 7.04 mmol) and allow the reaction mixture to warm slowly to room temperature. After 24 h concentrate the solution *in vacuo* and dissolve the viscous residue in ethyl acetate (100 ml), then pour into a separatory funnel containing distilled water (150 ml). Separate the phases and extract the aqueous layer with ethyl acetate (3 × 50 ml). Wash the combined organic extracts with saturated aqueous NaCl solution (200 ml), dry (MgSO<sub>4</sub>), filter off the drying agent and remove the solvent *in vacuo* to furnish the crude product. Purify by flash column chromatography (ethyl acetate) to provide ethyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio- $\alpha$ -D-galactopyranoside as a white foam (2.58 g, 88%).  $[\alpha]_D = +135.2$  (*c* 0.8, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.05 (s, (CH<sub>3</sub>)<sub>3</sub>CSi, 9H), 1.26 (t, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>, 3H), 2.55 (dq, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>, 1H), 2.62 (dq, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>, 1H), 3.54 (dd, *J* = 3.3 and 9.9 Hz, H-3, 1H), 3.70 (dd, *J* = 5.2 and 10.7 Hz, H-6, 1H), 3.91 (dd, *J* = 5.5 and 10.7 Hz, H-6, 1H), 4.11 (dd, *J* = 3.3 and 5.5 Hz, H-2, 1H), 4.12 (dd, *J* = 5.5 and 9.9 Hz, H-4, 1H), 4.17 (dd, *J* = 5.5 and 5.5 Hz, H-5, 1H), 5.42 (d, *J* = 5.5 Hz, H-1, 1H), 7.40 (m, ArH, 6H), 7.65 (m, ArH, 4H).

### Method 13 [99]

Preparation of methyl-4,6-*O*-(1,1,3,3-tetra-isopropylidisiloxanylidene)-1-thio- $\alpha$ -D-mannopyranoside.



TIPDSCl<sub>2</sub>, imidazole, 0 °C, 30 min.

#### Notes.

Kinetic double silylation of the 4,6-diol under mildly basic conditions initiated by reaction at the sterically most available 6-OH.

#### Materials.

1,3-Dichloro-1,1,3,3-tetra-isopropylidisiloxane (5.44 ml, 17.0 mmol)	corrosive
Methyl-1-thio- $\alpha$ -D-mannopyranoside (3.00 g, 14.3 mmol)	assume toxic
Imidazole (3.87 g, 56.8 mmol)	harmful, corrosive
Acetonitrile (anhydrous, 60 ml)	highly flammable, toxic
Methanol (10 ml)	highly flammable, toxic
Celite <sup>®</sup>	harmful by inhalation, possible irreversible effects
Toluene (few ml)	highly flammable, harmful, irritant
Ethyl acetate (chromatography solvent)	highly flammable, irritant
Hexane (chromatography solvent)	highly flammable, harmful

#### Equipment.

Round bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  
 Source of inert gas  
 Syringes (50 and 10 ml)  
 Magnetic stirrer  
 Ice bath  
 Column chromatography equipment

**Procedure.** Add 1,3-dichloro-1,1,3,3-tetra-isopropylidisiloxane (5.44 ml, 17.0 mmol) to a stirred solution of methyl-1-thio- $\alpha$ -D-mannopyranoside (3.00 g, 14.3 mmol) and imidazole (3.87 g, 56.8 mmol) in anhydrous acetonitrile (60 ml) cooled to 0 °C. Stir the solution at 0 °C for 30 min, then quench the reaction by the addition of methanol (10 ml) and filter the mixture through a pad of Celite<sup>®</sup>. Concentrate the filtrate *in vacuo* and azeotrope the residue with toluene. This is achieved by diluting the crude product with toluene (~30 ml) and concentrating the mixture *in vacuo*. Repeat this process several times. Purification of the crude product by flash column chromatography (3:17, ethyl acetate/hexane) affords methyl-4,6-*O*-(1,1,3,3-tetraisopropylidisiloxanylidene)-1-thio- $\alpha$ -D-mannopyranoside (5.50 g, 85%). [ $\alpha$ ]<sub>D</sub> = +85.3 (*c* 1.46, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.04–1.12 (m, 2 × [(CH<sub>3</sub>)<sub>2</sub>CH]<sub>2</sub>Si, 28H), 2.11 (s, SCH<sub>3</sub>, 3H), 2.44 (d, *J* = 5.3 Hz, OH, 1H), 2.55 (d, *J* = 4.0 Hz, OH, 1H), 3.82–3.91 (m, H-3, H-5, H-6, 3H), 4.06–4.19 (m, H-2, H-4, H-6, 3H), 5.26 (s, H-1, 1H).

#### Method 14 [100]

Preparation of methyl-2,3,4-tri-*O*-acetyl- $\alpha$ -D-mannopyranoside.



HF-pyridine, 0 °C → rt, 24 h.

#### Notes.

Selective desilylation leaving acid- and base-labile functionality intact.

Reactions involving hydrogen fluoride performed in ordinary laboratory glassware will lead to etching of the glass surface and possible weakening of the vessel (generating an implosion risk if placed under vacuum). It is therefore recommended that polyethylene containers be used; a photographic film cartridge fitted with a rubber septum makes an ideal reaction vessel for small scale work.

#### Materials.

Methyl-2,3,4-tri- <i>O</i> -acetyl-6- <i>O</i> - <i>tert</i> -butyldimethylsilyl- $\alpha$ -D-mannopyranoside (5.57 g, 12.8 mmol)	assume toxic
Tetrahydrofuran (anhydrous, 100 ml)	highly flammable, irritant
Hydrogen fluoride-pyridine complex (5.00 ml)	corrosive, toxic
Diethyl ether (50 ml)	extremely flammable, harmful
Saturated solution of sodium hydrogen carbonate (~50 ml)	no risk

Sodium hydrogen carbonate	no risk
Saturated solution of sodium chloride ( $3 \times 100$ ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Toluene (few ml)	highly flammable, harmful, irritant
Hexane (chromatography solvent)	highly flammable, harmful
Diethyl ether (chromatography solvent)	extremely flammable, harmful

#### Equipment.

Polyethylene vial (250 ml) with rubber septum and magnetic stirrer bar  
Source of inert gas  
Syringes (50 and 5 ml)  
Magnetic stirrer  
Ice bath

*Procedure.* Place methyl-2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl- $\alpha$ -D-manopyranoside (5.57 g, 12.8 mmol) and anhydrous THF (100 ml) in a dry polyethylene vial (250 ml) equipped with a rubber septum and magnetic stirring bar. Cool the solution to 0 °C, then treat with hydrogen fluoride–pyridine complex (5.00 ml, ~70% HF as a solution in pyridine). After 1 h remove the ice bath and stir the reaction at room temperature for 24 h. Dilute the solution with diethyl ether (50 ml), cool to 0 °C then quench the reaction by the dropwise addition of saturated aqueous NaHCO<sub>3</sub> solution (~50 ml), followed by solid NaHCO<sub>3</sub> until no more carbon dioxide is liberated. Transfer the mixture to a separatory funnel and wash the organic layer with saturated aqueous NaCl solution ( $3 \times 100$  ml), then dry (MgSO<sub>4</sub>), filter off the drying agent and remove the solvent *in vacuo* to furnish a pale yellow syrup. The crude product should be azeotroped with toluene to remove traces of pyridine and the resulting pale yellow crystals recrystallised from hexane/diethyl ether to furnish the product (3.78 g, 92%). Mp 97.0–99.6 °C;  $[\alpha]_D^{20} = +54.9$  (*c* 1.0, chloroform); *R*<sub>f</sub> 0.32 (diethyl ether); IR (KBr disc)/cm<sup>-1</sup> 3508 br (O–H), 2940 m (C–H), 2837 m (C–H), 1748 s (C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.93, 2.01 and 2.08 (3 × s, 3 × OAc, 9H), 3.34 (s, OCH<sub>3</sub>, 3H), 3.54–3.57 (m, 2 × H-6, 2H), 3.66–3.74 (m, H-5, 1H), 4.66 (d, *J* = 1.7 Hz, H-1, 1H), 5.17 (t, *J* = 10.1 Hz, H-4, 1H), 5.18 (dd, *J* = 1.7 and 3.3 Hz, H-2, 1H), 5.31 (dd, *J* = 3.3 and 10.1 Hz, H-3, 1H); MS *m/z* (CI), 338 (M + NH<sub>4</sub>, 15%), 321 (M + H, 6%), 289 (M – OMe, 100%).

*Method 15 [100]*

Preparation of methyl-2,3,4-tri-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl- $\alpha$ -D-mannopyranoside.



BzCl, pyridine, 0 °C  $\rightarrow$  rt, 18 h.

*Notes.*

Classical peracylation blocking all available hydroxyls.

*Materials.*

Benzoyl chloride (9.00 ml, 77.5 mmol)	corrosive
Methyl-6- <i>O</i> - <i>tert</i> -butyldimethylsilyl- $\alpha$ -D-mannopyranoside (3.42 g, 11.1 mmol)	assume toxic
Pyridine (anhydrous, 40 ml)	highly flammable, harmful
Saturated solution of sodium hydrogen carbonate (~ 100 ml)	no risk
Sodium hydrogen carbonate (~ 1.5 g)	no risk
Diethyl ether (3 $\times$ 50 ml)	extremely flammable, harmful
Hydrochloric acid (10% aqueous, 3 $\times$ 100 ml)	corrosive, toxic
Distilled water (100 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Toluene (few ml)	highly flammable, harmful, irritant
Hexane (chromatography solvent)	highly flammable, harmful
Diethyl ether (chromatography solvent)	extremely flammable, harmful

*Equipment.*

Round bottomed flask (250 ml) with rubber septum and magnetic stirrer bar  
 Source of inert gas  
 Syringes (50 and 10 ml)  
 Magnetic stirrer  
 Ice bath  
 Column chromatography equipment

*Procedure.* Slowly add benzoyl chloride (9.00 ml, 77.5 mmol) to a stirred solution of methyl-6-*O*-*tert*-butyldimethylsilyl- $\alpha$ -D-mannopyranoside (3.42 g, 11.1 mmol) in dry pyridine (40 ml) cooled to 0 °C. Allow the reaction mixture to warm gradually to room temperature. After 18 h the reaction will be complete by TLC analysis. Cool the flask to 0 °C and quench the reaction by the dropwise addition of saturated aqueous NaHCO<sub>3</sub> solution (~100 ml), followed by solid NaHCO<sub>3</sub> (~1.5 g). Extract the resulting solution with diethyl ether (3  $\times$  50 ml) then wash the ethereal layers with 10% aqueous hydrochloric acid (3  $\times$  100 ml) and distilled water (100 ml). Drying the organic layers (MgSO<sub>4</sub>) and removal of the solvent *in vacuo* affords the crude product. Residual traces of pyridine can be removed if the product is azeotroped with toluene. This is achieved by diluting the crude product with toluene (~30 ml) and concentrating the mixture *in vacuo*. This process should be repeated until the residue becomes highly viscous. Purification by flash column chromatography (3:2, hexane/diethyl ether) furnishes the product as a white foam (6.76 g, 98%).  $[\alpha]_D^{20} = +98.7$  (*c* 1.0, chloroform); *R*<sub>f</sub> 0.22 (3:2, hexane/diethyl ether); IR (liquid film)/cm<sup>-1</sup> 2930 m (C–H), 1732 s (C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 0.00 and 0.03 (2  $\times$  s, Si(CH<sub>3</sub>)<sub>2</sub>, 6H), 0.90 (s, (CH<sub>3</sub>)<sub>3</sub>CSi, 9H), 3.50 (s, OCH<sub>3</sub>, 3H), 3.84–3.38 (m, 2  $\times$  H-6, 2H), 4.06–4.16 (m, H-5, 1H), 4.97 (d, *J* = 1.7 Hz, H-1, 1H), 5.66 (dd, *J* = 1.7 and 3.3 Hz, H-2, 1H), 5.83 (dd, *J* = 3.3 and 10.1 Hz, H-3, 1H), 5.96 (t, *J* = 10.1 Hz, H-4, 1H), 7.20–7.63 and 7.80–7.98 (m, Ar-H, 15H); MS *m/z* (CI), 621 (M + H, 5%), 589 (61%, M<sup>+</sup> – OMe), 241 (13), 179 (21), 105 (100).

*Method 16 [101]*

Preparation of phenyl- $\alpha$ -D-glucopyranoside.



Cat. NaOMe, MeOH, rt, 24 h.

*Notes.*

Classical Zemplén acetate deprotection; under these conditions all acetate groups and most other esters are cleaved.



*Materials.*

Phenyl-2,3,4,6-tetra- <i>O</i> -acetyl-1- <i>O</i> - $\alpha$ -D-glucopyranoside (1.19 g, 2.80 mmol)	assume toxic
Dichloromethane (anhydrous, 10 ml)	toxic, irritant, possible irreversible effects
Methanol (anhydrous, 20 ml)	highly flammable, toxic
Solution of sodium methoxide in methanol (0.20 ml, 0.10 mmol, 0.5 M)	highly flammable, reacts violently with water, corrosive
Amberlite IR 120 (H <sup>+</sup> ) resin	irritant

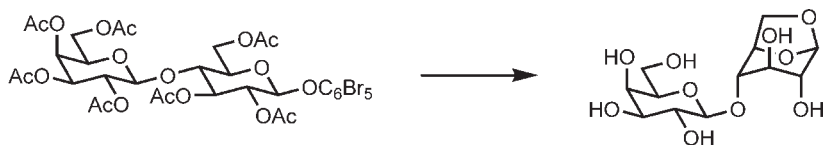
*Equipment.*

Round bottomed flask (50 ml) with rubber septum and magnetic stirrer bar  
 Source of inert gas  
 Syringes (10 ml, 20 ml and 200  $\mu$ l)  
 Magnetic stirrer

*Procedure.* Stir a solution of phenyl-2,3,4,6-tetra-*O*-acetyl-1-*O*- $\alpha$ -D-glucopyranoside (1.19 g, 2.80 mmol) in anhydrous dichloromethane (10 ml) and methanol (20 ml) at room temperature for 20 min. Add a solution of sodium methoxide (0.20 ml, 0.10 mmol, 0.5 M) and leave the reaction to stir until TLC analysis indicates that the starting material has been consumed ( $\sim$  24 h). Neutralize the reaction by the addition of Amberlite IR 120 (H<sup>+</sup>) resin. Stir for 10 min, then filter off the resin and concentrate the filtrate *in vacuo* to afford the deacetylated product as a yellow solid (0.69 g, 96%). IR (KBr disc)/cm<sup>-1</sup> 3473 br, 3421 br and 3300 br (O–H), 2925 s (C–H), 2900 s (C–H), 1601 s and 1590 s (C=C), 1497 vs, 1366 s and 1229 s (C–H), 1109 vs, 1035 vs and 1012 vs (C–O), 756 s and 691 s (Ar); <sup>1</sup>H NMR (CD<sub>3</sub>OD) 3.49 (dd, *J* = 9.0 and 9.5 Hz, H-4, 1H), 3.63 (dd, *J* = 2.0 and 9.5 Hz, H-2, 1H), 3.71–3.81 (m, H-5 and 2  $\times$  H-6, 3H), 3.92 (dd, *J* = 9.0 and 9.5 Hz, H-3, 1H), 5.53 (d, *J* = 2.0 Hz, H-1, 1H), 7.04–7.35 (m, ArH, 5H); MS *m/z* (CI), 274 (M + NH<sub>4</sub>, 8%), 180 (15), 94 (100).

*Method 17 [102]*

Preparation of 1,6-anhydro-( $\beta$ -galactopyranosyl)-(1'  $\rightarrow$  3)- $\beta$ -lactoside.



Amberlite IRA 400 (OH), MeOH, 50 °C, 5 h.

#### Notes.

Deacylation employing anionic exchange resin which facilitates isolation of the product by simple filtration.

Deprotection of the 1°-hydroxyl initiates formation of the intramolecular glycoside (1,6-anhydrosugar), resulting in simultaneous protection of the anomeric centre and the 6-OH.

Note the conformational change of the glucose unit (from  ${}^4C_1$  to  ${}^1C_4$ ) which places the 2- and 3-hydroxyls in less reactive axial sites.

#### Materials.

Amberlite IRA 400 (OH) resin (300 ml)	irritant
Hepta- <i>O</i> -acetyl-pentabromophenyl lactoside (30.0 g, 27.1 mol)	assume toxic
Methanol (anhydrous, 250 + 3 × 20 ml)	highly flammable, toxic

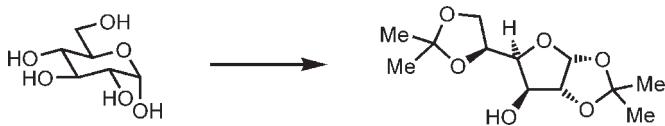
#### Equipment.

Round bottomed flask (1000 ml) with rubber septum and magnetic stirrer bar  
Source of inert gas  
Heater stirrer with thermostat thermometer  
Oil bath

**Procedure.** Add freshly activated Amberlite IRA 400 (OH) resin (300 ml) to a stirred suspension of hepta-*O*-acetyl-pentabromophenyl lactoside (30.0 g, 27.1 mol) in anhydrous methanol (250 ml). Stir the reaction at 50 °C for 5 h. After this time the reaction will have gone to completion by TLC analysis (3:7, dichloromethane/methanol). Filter the mixture and wash the resin with methanol (3 × 20 ml), then concentrate the filtrate *in vacuo* to furnish the product as a white powder (7.48 g, 85%).  ${}^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) 3.32–3.90 (m, 12H), 4.07 (d,  $J = 8.0$  Hz, H-1', 1H), 4.48 (d,  $J = 8.0$  Hz, H-1, 1H); MS  $m/z$  (ES) 347 ( $\text{M} + \text{Na}$ , 5%), 179 ( $\text{M} - \text{O}[\beta\text{-D-glucopyranose}]$ , 14%), 145 ( $\text{M} - \text{O}[\beta\text{-D-galactopyranose}]$ , 12%).

#### Method 18 [103]

Preparation of 1,2,5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose.



Acetone, cat. I<sub>2</sub>, rt, 4 h.

### Notes.

Straightforward preparation of diacetone glucose.

### Materials.

D-Glucose (1.00 g, 5.55 mmol)	no known risk
Iodine (0.30 g, 1.18 mmol)	corrosive, harmful
Acetone (50 ml)	highly flammable
Solution of sodium thiosulfate (5% aqueous, ~10 ml)	irritant
Chloroform (3 × 20 ml)	harmful, irritant, possible irreversible effects
Distilled water (100 ml)	no risk
Sodium sulfate (drying agent)	irritant
Diethyl ether (recrystallisation solvent)	extremely flammable, harmful
Petroleum ether (recrystallisation solvent)	highly flammable, harmful

### Equipment.

Round bottomed flask (100 ml) with magnetic stirrer bar  
Syringe (50 ml)  
Magnetic stirrer

**Procedure.** Add D-glucose (1.00 g, 5.55 mmol) to a solution of iodine (0.30 g, 1.18 mmol) in acetone (50 ml) and stir the suspension at room temperature for 4 h. After this time the sugar will have dissolved completely and the reaction will have gone to completion by TLC analysis (19:1, chloroform/methanol). Quench the reaction by the addition of dilute sodium thiosulfate solution to render the reaction mixture colourless, then remove the acetone *in vacuo*. Transfer the aqueous solution to a separatory funnel and extract with chloroform (3 × 20 ml), then wash the combined organic layers with distilled water (100 ml), dry (Na<sub>2</sub>SO<sub>4</sub>) and remove solvents *in vacuo* to afford the crude product. Recrystallisation (diethyl ether/petrol) affords di-acetone glucose as colourless crystals (1.09 g, 80%). Mp 109–110 °C; [ $\alpha$ ]<sub>D</sub> = –13.5 (c 1.0, chloroform); NMR (CDCl<sub>3</sub>) 1.32, 1.37, 1.45 and 1.50 (4 × s, 4 × CH<sub>3</sub>,

12H), 2.68 (d,  $J = 3.2$  Hz, OH, 1H), 3.99 (dd,  $J = 5.6$  and 8.8 Hz, H-6, 1H), 4.07 (dd,  $J = 2.8$  and 8.0, H-4, 1H), 4.17 (dd,  $J = 6.0$  and 8.8 Hz, H-6, 1H), 4.32–4.37 (m, H-3 and H-5, 2H), 4.53 (~d,  $J = 3.7$  Hz, H-2, 1H), 5.94 (d,  $J = 3.7$  Hz, H-1, 1H).

#### Method 19 [104]

Preparation of methyl-3-*O*-allyl-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranoside.



2,2-DMP, cat. TsOH, rt, 20 min.

#### Notes.

Alternative preparation of isopropylidene derivatives under mild conditions leaving the anomeric centre unaffected.

#### Materials.

<i>para</i> -Toluenesulfonic acid monohydrate (100 mg, 0.53 mmol)	irritant
Methyl-3- <i>O</i> -allyl- $\beta$ -D-glucopyranoside (1.00 g, 4.27 mmol)	assume toxic
2,2-Dimethoxypropane (5.00 ml, 40.7 mmol)	highly flammable, harmful, irritant
Sodium hydrogen carbonate (~50 mg)	no risk
Dichloromethane (50 ml)	toxic, irritant, possible irreversible effects
Distilled water (2 $\times$ 25 ml)	no risk
Solution of saturated aqueous sodium chloride (50 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful by inhalation
Hexane (chromatography solvent)	highly flammable, harmful
Ethyl acetate (chromatography solvent)	highly flammable, irritant

#### Equipment.

Round bottomed flask (10 ml) with magnetic stirrer bar  
Syringe (5 ml)

Magnetic stirrer

Column chromatography equipment

*Procedure.* Add *para*-toluenesulfonic acid monohydrate (100 mg, 0.53 mmol) to a stirred suspension of methyl-3-*O*-allyl- $\beta$ -D-glucopyranoside (1.00 g, 4.27 mmol) in 2,2-dimethoxypropane (5.00 ml, 40.7 mmol). Stir the solution for 20 min at room temperature, then quench the reaction by the addition of solid NaHCO<sub>3</sub> (~50 mg). Dilute the solution with dichloromethane (50 ml), transfer to a separatory funnel and wash the organic phase successively with water (2  $\times$  25 ml) and saturated aqueous NaCl solution (50 ml). Dry the organic phase (MgSO<sub>4</sub>), filter off the drying agent and concentrate the solution *in vacuo* to afford a syrup. Purification of the crude product by flash column chromatography (6:4, hexane/ethyl acetate) affords methyl-3-*O*-allyl-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranoside (1.08 g, 92%). [ $\alpha$ ]<sub>D</sub> = -28.5 (*c* 0.34, chloroform); *R*<sub>f</sub> 0.51 (6:4, hexane/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.42 and 1.50 (2  $\times$  s, (CH<sub>3</sub>)<sub>2</sub>C, 6H), 2.73 (br, OH, 1H), 3.26 (m, H-5, 1H), 3.43 (m, 2  $\times$  H-6, 2H), 3.56 (s, OCH<sub>3</sub>, 3H), 3.65 (m, H-2, 1H), 3.78 (dd, *J* = 10.0 and 10.0 Hz, H-3, 1H), 3.93 (dd, *J* = 5.5 and 10.0 Hz, H-4, 1H), 4.28 (d, *J* = 7.5 Hz, H-1, 1H), 4.31 (m, OCH<sub>2</sub>-CH=CH<sub>2</sub>, 2H), 5.25 (m, -CH=CH<sub>2</sub>, 2H), 5.95 (m, -CH=CH<sub>2</sub>, 1H).

#### Method 20 [101]

Preparation of phenyl-4,6-*O*-benzylidene-1-seleno- $\beta$ -D-glucopyranoside.



PhCH(OMe)<sub>2</sub>, cat. CSA, 45–50 °C, 20 mm Hg.

#### Notes.

The experiment is performed on a rotary evaporator in order to drive the equilibrium process to completion (by continual removal of liberated methanol). Because the product is somewhat unstable on silica gel, a small quantity of triethylamine is added to neutralise the slurry and minimise decomposition during column chromatography.

The difficulty of transferring the product onto the column as a viscous oil or syrup is overcome by employing the 'dry loading' technique. The product is preabsorbed onto silica gel by dissolving the syrup in a minimum volume of solvent, adding dry silica gel and removing the solvent *in vacuo*. The resulting freely flowing powder is easily loaded onto the chromatography column and mechanical losses are kept to a minimum.

*Materials.*

Camphor sulfonic acid (0.79 g, 3.39 mmol)	corrosive
Benzaldehyde dimethyl acetal (2.80 ml, 18.7 mmol)	irritant
Phenyl-1-seleno- $\beta$ -D-glucopyranoside (5.42 g, 17.0 mmol)	assume toxic
<i>N,N</i> -dimethylformamide (anhydrous, 80 ml)	irritant, harmful
Triethylamine (2.40 ml, 17.0 mmol)	highly flammable, corrosive, harmful
Toluene (few ml)	highly flammable, harmful, irritant
Dichloromethane (few ml)	toxic, irritant, possible irreversible effects
Ethyl acetate (chromatography solvent)	highly flammable, irritant

*Equipment.*

Round bottomed flask (250 ml)

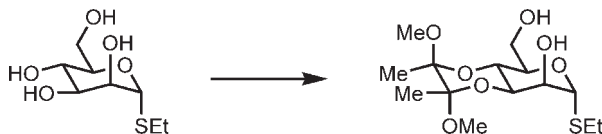
Rotary evaporator

Column chromatography equipment

*Procedure.* Add camphor sulfonic acid (0.79 g, 3.39 mmol) and benzaldehyde dimethyl acetal (2.80 ml, 18.7 mmol) to a solution of phenyl-1-seleno- $\beta$ -D-glucopyranoside (5.42 g, 17.0 mmol) in anhydrous DMF (80 ml). Place the resultant mixture on a rotary evaporator at a pressure of 20 mm Hg and a temperature of 45–50 °C. When TLC analysis indicates that the reaction is complete, add triethylamine (2.40 ml, 17.0 mmol) and concentrate the solution *in vacuo* employing toluene to azeotropically remove residual DMF. Dissolve the crude oil in a minimum volume of a 1% triethylamine solution in dichloromethane preadsorbed onto silica gel, then submit this mixture to flash column chromatography using gradient elution (8:1, toluene/ethyl acetate  $\rightarrow$  ethyl acetate + 1% triethylamine) to afford the product as an orange solid (6.13 g, 89%). Mp 162–164 °C;  $[\alpha]_{\text{D}}^{28} = -44.9$  (*c* 0.74, dichloromethane); IR (KBr disc)/cm<sup>-1</sup> 3456 br and 3269 br (O–H), 3071 s and 3028 s (C–H), 2970 vs and 2946 s (C–H), 2877 vs and 2845 s (C–H), 1463 s, 1455 s and 1440 w (C=C), 1377 s (C–H), 1107 s, 1085 s and 1058 s (O–H), 1040 w and 1006 s (C–O), 741 w and 691 w (Ar); <sup>1</sup>H NMR (CD<sub>3</sub>OD) 3.29–3.48 (m, H-2, H-4, H-5, 3H), 3.62 (~t, *J* = 8.5 and 9.0 Hz, H-3, 1H), 3.72 (~t, *J* = 10.0 Hz, H-6, 1H), 4.24 (dd, *J* = 4.5 and 10.0 Hz, H-6, 1H), 4.89 (d, *J* = 10.0 Hz, H-1, 1H), 5.54 (s, PhCH, 1H), 7.28–7.66 (m, Ar-H, 10H). MS *m/z* (CI) 409 (M + H, 6%), 251 (100), 235 (35), 129 (37), 105 (53), 78 (30).

*Method 21 [105]*

Preparation of (2'*S*,3'*S*)-ethyl-3,4-*O*-[2',3'-dimethoxybutan-2',3'-diyl]-1-thio- $\alpha$ -D-mannopyranoside.



Butanedione,  $\text{CH}(\text{OMe})_3$ , cat. CSA,  $\Delta$ , 19 h.

*Notes.*

Selective protection of a *trans*-diequatorially disposed diol as a butane-1,2-diacetal (BDA derivative).

Cyclohexane-1,2-diacetal (CDA) and dispiroketal (dispoke) protocols provide bulkier groups for *trans*-1,2-diol protection [86].

*Materials.*

Trimethylorthoformate (25.0 ml, 229 mmol)	highly flammable, irritant
Butane-2,3-dione (7.50 ml, 85.5 mmol)	highly flammable, harmful, irritant
Ethyl-1-thio- $\alpha$ -D-mannopyranoside (12.0 g, 53.5 mmol)	assume toxic
Camphor sulfonic acid (1.44 g, 6.20 mmol)	corrosive
Methanol (anhydrous, 150 ml)	highly flammable, toxic
Triethylamine (1.00 ml)	highly flammable, corrosive, harmful
Petroleum ether (chromatography solvent)	highly flammable, harmful
Diethyl ether (chromatography solvent)	extremely flammable, harmful

*Equipment.*

Two-necked round bottomed flask (500 ml) with rubber septum and magnetic stirrer bar

Reflux condenser

Source of inert gas

Syringes (50 and 10 ml)  
Heater stirrer with thermostat thermometer  
Column chromatography equipment

*Procedure.* Add successively trimethylorthoformate (25.0 ml, 229 mmol) and butane-2,3-dione (7.50 ml, 85.5 mmol) to a stirred solution of ethyl-1-thio- $\alpha$ -D-mannopyranoside (12.0 g, 53.5 mmol) and camphor sulfonic acid (1.44 g, 6.20 mmol) in anhydrous methanol (150 ml). Heat the reaction mixture at reflux for 19 h. Cool the solution to room temperature and quench the reaction by the addition of triethylamine (1.00 ml), then concentrate the solution *in vacuo* to afford a red oil. Purification by flash column chromatography using gradient elution (1:1, petrol/diethyl ether  $\rightarrow$  diethyl ether) gives the BDA-protected product as a foam (14.7 g, 81%).  $[\alpha]_D^{27} = -299$  (c 1.05, chloroform); IR (liquid film)/ $\text{cm}^{-1}$  3442 (O–H), 2950 (C–H), 1455, 1132;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.24–1.31 (m,  $2 \times \text{CH}_3$ ,  $\text{SCH}_2\text{CH}_3$ , 9H), 2.20 (t,  $J = 6.5$  Hz, OH, 1H), 2.51–2.75 (m,  $\text{SCH}_2\text{CH}_3$ , 2H), 2.97 (d,  $J = 1.9$  Hz, OH, 1H), 3.25 (s,  $\text{OCH}_3$ , 3H), 3.26 (s,  $\text{OCH}_3$ , 3H), 3.79–3.85 (m,  $2 \times \text{H-6}$ , 2H), 3.93–4.01 (m, H-2 and H-3, 2H), 4.11–4.14 (m, H-4 and H-5, 2H), 5.31 (s, H-1, 1H); MS  $m/z$  (FAB) 337 (M–H, 68%), 323 (58), 307 (M–OMe, 84%), 275 (24), 245 (20), 189 (30), 171 (59), 145 (22), 127 (60), 116 (100).

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# Synthesis and Activation of Carbohydrate Donors: Acetates, Halides, Phenyl selenides and Glycals

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## 3.1 INTRODUCTION

This chapter deals with a mixed bag of glycosyl donor systems whose glycosylating reactivities rely on a range of differing mechanisms that exploit familiar leaving group chemistries (bromides, chlorides and acetates) on the one-hand to less familiar leaving groups (fluorides and phenylselenides) or electrophiles (1,2-anhydride/ glycosyl epoxides formed *in situ* from glycals) on the other.

Of these various donors, peracetylated derivatives of glycosyl bromides (the so-called acetobromoglycoses) continue to be the most popular by virtue of their long history of use (and hence wealth of available literature stretching back to the pioneering work of Königs and Knorr [1]) their commercial availability and relative ease of preparation. While the utility of bromides is undeniable, the other less frequently used donors in this chapter offer certain advantages in terms of convenience and strategy.

## 3.2 ACETATES

Anomeric acetates, typically peracetylated carbohydrates, are frequently encountered as intermediates in the preparation of other glycosyl donors, including glycosyl

halides. Although they are less reactive than many other donors, they can in fact be used as effective glycosyl donors in their own right and may, as we shall see, offer certain strategic advantages.

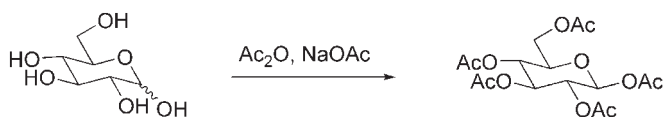
### 3.2.1 Preparation of acetates

The preparation of acetates is a straightforward process that is most conveniently achieved using acetic anhydride [2]. Different anomers may be formed stereoselectively simply through variation of reaction conditions: the use of NaOAc as a weak base in neat  $\text{Ac}_2\text{O}$  exploits the kinetic anomeric effect and results in exclusive  $\beta$ -anomer formation [3], whereas the use of a strong, hard Lewis acid, such as  $\text{ZnCl}_2$ , allows equilibration and results in the formation of the thermodynamic  $\alpha$ -product [4]. For many purposes, an anomeric mixture of acetates will suffice and in these cases they can be produced simply by dissolving the free sugar in a near equal v/v solution of  $\text{Ac}_2\text{O}$ /pyridine followed by removal of the solvent and purification [5]. As a nice refinement, the use of toxic pyridine can be avoided by using iodine-catalysed acetylation as a recently proposed mild variation [6].

Peracetylated furanose derivatives, are typically inaccessible by these methods but some novel methods, including the first example of a crystalline peracetylated furanose [7] have recently been disclosed.

#### Method 1

The synthesis of  $\beta$ -glucopyranose pentaacetate using sodium acetate [3, 4, 8].



*Notes and discussion.* This method is based on one first used almost 125 years ago but is nonetheless highly effective. The use of a weak base (NaOAc) sets up conditions of kinetic control which, as a result of the greater nucleophilicity of the  $\beta$ -anomer of glucose, give rise to the  $\beta$ -peracetate product.

#### Materials.

D-Glucose (100 g, 454 mmol)	no known risk
Sodium acetate (50 g, 609 mmol)	irritant

Acetic anhydride (700 ml)	flammable, corrosive, reacts violently with water, lachrymator, toxic
Ice or ice water (2 l)	no risk
Ethanol	flammable, toxic

*Equipment.*

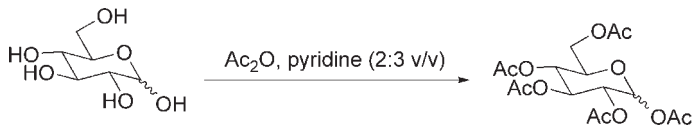
Round bottom flask (2 l)  
Heater stirrer with thermostat thermometer (and/or heat gun)  
Oil bath  
Filtration apparatus  
Conical flask (2 l)

*Special precautions.* This reaction is exothermic and it is particularly important that when performed on the scale described here the sugar is added portion-by-portion to the base/anhydride suspension. Once the reaction starts to boil, without the aid of a heat source, then boiling can be maintained by frequent additions of small sugar portions. Since this stage involves the boiling of acetic anhydride, some workers may wish to use a condenser, however this can hamper the frequent sugar additions. A heat gun can provide a very convenient source of rapidly controllable heat for the initial stages of the reaction. If the final recrystallization is left for too long then some  $\alpha$ -peracetate may also crystallize and it is typically best to filter as soon as the ethanolic solution has reached room temperature.

*Procedure.* Stir a suspension of anhydrous sodium acetate (50 g, 609 mmol) and acetic anhydride (700 ml) in a fitted 2 l round bottom flask and gently heat to boiling. Add about 3 g of the D-glucose (50 g in total, 454 mmol) and continue to gently heat. The reaction will begin to reflux gently under its own heat (this may require addition of further small portions of sugar) and at this point the flask should be removed from the heat source and reflux maintained by repeated additions of small sugar portions with occasional agitation to prevent excessive build-up of unreacted sugar on the bottom of the flask. After all the sugar has been added and the resulting solution is no longer exothermic then it should be heated at full boil for 30 min. After cooling, pour the solution onto the ice and stand for 2 h with occasional scratching and stirring to encourage crystallization and to prevent the formation of a block of solid, respectively. Collect the solid formed (~150–175 g) by filtration and wash with cold water. Purify by recrystallization from hot EtOH (1 l, 95%). Mp = 132–133 °C;  $[\alpha]_D^{24} = +5$  (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz): 5.70 (d, 1H, H-1, *J*<sub>1,2</sub> = 7.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 50 MHz): 91.6 (C-1), 61.2 (C-6) 67.5, 70.0, 72.5, 72.6 (C-2–C-5).

*Method 2*

The synthesis of  $\alpha,\beta$ -D-glucopyranose pentaacetate using pyridine [4, 5, 9].



*Notes and discussion.* This is a rapid esterification, which consequently gives rise to a mixture of anomeric acetates that are formed more rapidly than mutarotation can take place. The reaction is relatively mild and yields peracetylated products that have convenient solubilities and chromatographic mobilities and so can also be used as an indirect method for isolating carbohydrates from potentially difficult mixtures. The major disadvantage of the method, other than the formation of an anomeric mixture, is the odour, toxicity and low volatility of pyridine, which is used as co-solvent. Although here the product is obtained by crystallization, quantitative yields may be achieved effectively if flash chromatography is used (EtOAc with cyclohexane/hexane is a typically good eluant system). In this case, it is advisable to ensure that as much pyridine has been removed from the crude mixture as possible [shaking with HCl (aq., 2 M) and removal of pyridine *in vacuo*] prior to chromatography.

*Materials.*

D-Glucose (10 g, 45 mmol)	no known risk
Acetic anhydride (50 ml)	flammable, corrosive, reacts violently with water, lachrymator, toxic
Pyridine (75 ml, distilled from $\text{CaH}_2$ or BaO)	flammable, harmful, toxic
Ice water (200 ml)	no risk
Ethanol	flammable, toxic

*Equipment.*

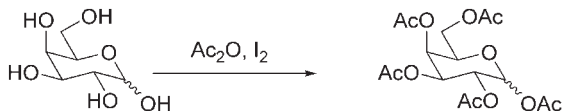
Round bottom flask (250 ml)  
Ice bath  
Conical flask (500 ml)  
Filtration apparatus

*Special precautions.* Gloves, lab. coat and goggles should be worn at all times. Pyridine is toxic and readily absorbed through the skin: wear suitably protective gloves and conduct the reaction in a fumehood.

*Procedure.* Cool the 3:2 (v/v) solution of pyridine (75 ml)/acetic anhydride (50 ml) with an ice bath under nitrogen. Add the D-glucose and stir the suspension until the sugar dissolves. Allow the solution to warm to room temperature and stir for a further 16 h under nitrogen. After this time, pour the solution into ice water (200 ml);  $\alpha$ -D-glucopyranose pentaacetate will crystallize out rapidly—later crops yield  $\beta$ -D-glucopyranose pentaacetate.  $\alpha$ -D-Glucopyranose pentaacetate may be recrystallized from ethanol (19.4 g, 90%); mp 113 °C;  $[\alpha]_D^{24} = +105$  (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz): 6.35 (d, 1H, H-1,  $J_{1,2} = 3.6$  Hz).

### Method 3

The synthesis of  $\alpha,\beta$ -D-galactopyranose pentaacetate using iodine as a Lewis acid [6].



*Notes and discussion.* This modern variation provides a nice, mild alternative to pyridine/acetic anhydride. Here the small amount of  $\beta$ -anomer formed can be removed by recrystallization from ethanol.

### Materials.

D-Galactose (1 g, 5.6 mmol)	no known risk
Iodine (50 mg)	irritant
Acetic anhydride (5 ml)	flammable, corrosive, reacts violently with water, lachrymator, toxic
Ice (25 ml)	no risk
Dichloromethane (50 and 2 × 50 ml)	risk of irreversible effects
Solution of sodium thiosulfate (aq. dil.) (25 ml)	harmful
Solution of sodium carbonate (1 M, 25 ml)	corrosive, harmful
Magnesium sulfate (drying agent)	irritant, harmful



*Equipment.*

Round bottom flask (25 ml)  
Separating funnel (250 ml)  
Conical flask (100 ml)  
Filtration apparatus  
Rotary evaporator

*Special precautions.* The reaction is exothermic and on a large scale the sugar should be added portion by portion to a solution of iodine in acetic anhydride.

*Procedure.* Suspend and stir the D-galactose (1 g, 5.6 mmol) in acetic anhydride (5 ml) in a 25 ml round bottom flask. Add the iodine (50 mg). The suspended sugar should dissolve in the solution within 5 min to yield a dark yellow solution. After 20 min pour the solution into a separating funnel (250 ml) containing dichloromethane (50 ml), sodium thiosulfate solution (25 ml, dil. aq.) and crushed ice (25 ml) and shake until the organic layer is decolourised. Separate the organic layer, wash with sodium carbonate solution (25 ml), dry over magnesium sulfate, filter and remove the solvent *in vacuo*. The peracetylated galactose, as a 95:5  $\alpha/\beta$  mixture of anomers, will crystallize out as the solvent is removed; mp 95 °C;  $[\alpha]_D^{23} = +105$  (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 2.02, 2.04, 2.15, 2.18 (s  $\times$  4, 3H  $\times$  4, Ac  $\times$  3), 4.08 (dd, 1H, H-6, J = 5.8, 12.0 Hz), 4.13 (dd, 1H, H-6, J = 6.6, 12.0 Hz), 4.35 (dd, 1H, H-5, J = 5.8, 6.6 Hz), 5.34 (m, 2H, H-2,3), 6.38 (d, 1H, H-1, J = 1.2 Hz).

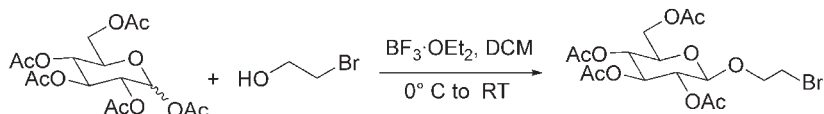
### 3.2.2 Use of anomeric acetates as glycosyl donors

Under the influence of strong, hard Lewis acid catalysis, the anomeric acetate can serve as an effective leaving group and therefore allow glycosylation. Indeed, acetates act as glycosyl donors during the preparation of e.g. glycosyl bromides (*vide infra*, here HBr acts both a source of Br<sup>−</sup> and as an acid catalyst) or thioglycosides (here BF<sub>3</sub>·OEt<sub>2</sub> is used as the Lewis acid).

Clearly, the major consideration is the need for acid-stability in both glycosyl donor and acceptor. In fact, prolonged reaction times may lead to associated acid-catalysed A<sub>Ac</sub>2 acetate hydrolysis in the acetylated donor or in the acetylated glycosylation products. Note too the use of a large excess of Lewis acid. Although the role of the Lewis acid is as a catalyst, peracetate glycosyl donors (and possibly glycosyl acceptors also) contain a large number of potentially Lewis basic sites that may sequester a given activator.

*Method 4*

The synthesis of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucoside from D-glucose pentaacetate [10, 11].



*Notes and discussion.* Note the use of 5 equiv. of boron trifluoride etherate in this procedure. Use of fewer equivalents (<5 equiv.) results in a sluggish, low yielding reaction.

Here the glycosyl acceptor contains a group (bromide) that is incompatible with the use of a soft Lewis acid activator and so precludes the use of some alternative glycosylation donors, such as glycosyl bromides or thioglycosides. As a consequence of anchimeric assistance by the C-2 acetate group, the  $\beta$ -glucoside is the major product.

*Materials.*

D-Glucose pentaacetate (2 g, 5.1 mmol)	treat as toxic
2-Bromoethanol (distilled from CaO, 0.45 ml, 6.3 mmol)	treat as toxic
Dichloromethane (9 ml distilled from CaH <sub>2</sub> )	highly flammable, harmful
Dichloromethane (15 ml $\times$ 3)	risk of irreversible effects
BF <sub>3</sub> ·OEt <sub>2</sub> (3.3 ml, 26.0 mmol)	flammable, corrosive
Ice water (15 ml $\times$ 2)	no risk
Water (15 ml)	no hazard
Sodium hydrogencarbonate solution (aq., saturated, 15 ml)	assume toxic
Magnesium sulfate	irritant
Ethyl acetate for chromatography	flammable irritant
Hexane for chromatography	toxic, flammable, irritant
Iso-octane for recrystallization	toxic, flammable, irritant

*Equipment.*

Round bottomed flask (25 ml)  
Syringe (5 ml) and needle  
Nitrogen source/balloon and bubbler head/septum  
Ice bath  
Conical flask (100 ml  $\times$  2)  
Separatory funnel (250 ml)  
Filtration equipment  
Rotary evaporator  
Flash chromatography equipment

*Special precautions.* Safety glasses and gloves should be worn during this experiment and work should be carried out in a fume hood:  $\text{BF}_3 \cdot \text{OEt}_2$ , in particular, is corrosive. Take care not to add the  $\text{BF}_3 \cdot \text{OEt}_2$  too rapidly—the ‘hot spots’ formed in the solution result in decomposition and lower yields. Do not prolong the reaction time unnecessarily as this leads to the deacetylation of the product 2-bromoethyl glucoside.

*Procedure.* Dissolve the D-glucose pentaacetate (2 g, 5.1 mmol) and 2-bromoethanol (0.45 ml, 6.3 mmol) in DCM (9 ml) under nitrogen and cool to 0 °C using the ice bath. Add the freshly distilled  $\text{BF}_3 \cdot \text{OEt}_2$  (3.3 ml, 26.0 mmol) dropwise using the syringe and needle over the course of 15 min. After 1.5 h allow the solution to warm to room temperature. After 20 h pour the reaction solution into ice water (15 ml) and extract with DCM three times (15 ml  $\times$  3). Combine these organic extracts, wash with water (15 ml), sat.  $\text{NaHCO}_3$  solution (aq., 15 ml), water again (15 ml) and then dry over magnesium sulfate. Filter the dried solution and remove the solvent on the rotary evaporator. Purify the resulting residue by flash chromatography (EtOAc/hexane, 1:3) to give 2-bromoethyl 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranoside (1.42 g, 61%) as a white solid, which can be recrystallised from ethyl acetate/isooctane; mp 118–120 °C (lit. [12] mp 117.3 °C (EtOH));  $[\alpha]_{\text{D}}^{27} = -11.9$  (c 1.65,  $\text{CHCl}_3$ ) (lit. [77]:  $[\alpha]_{\text{D}}^{20} = -12.3$  (c 0.2,  $\text{CHCl}_3$ ));  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) 2.00, 2.02, 2.07, 2.09 (s  $\times$  4, 3H  $\times$  4, Ac  $\times$  4), 3.42–3.51 (m, 2H), 3.67–3.87 (m, 2H), 4.10–4.31 (m, 3H), 4.57 (d,  $J_{1,2} = 8$  Hz, 1H, H-1), 4.97–5.27 (m, 3H).

### 3.3 HALIDES

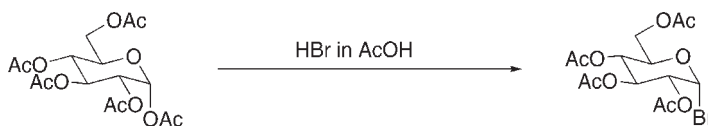
This section is considered in two separate themes that reflect the very differing modes of reactivity of glycosyl bromides/chlorides, on one hand, and glycosyl fluorides on the other. Although the use of glycosyl iodides has been recently highlighted [13, 14], their much higher reactivities compared with the other halides typically necessitate *in situ* preparation. This, combined with elimination and *in situ* anomerization with sugar alkoxides or simple alcohol glycosyl acceptors, reduces selectivity. These properties, at present, appear to somewhat restrict their general utility. (For an exception, see the very useful preparation of L-fucosyl nucleotidediphosphate in Chapter 12) [14].

### 3.3.1 Preparation of glycosyl bromides and chlorides

Glycosyl bromides and glycosyl chlorides may be most readily prepared by action of a source of dry hydrogen halide either added directly, as for glycosyl bromides, or generated *in situ*, as for glycosyl chlorides. The activity of these haloethers as glycosyl donors is high and so, unlike their fluoride counterparts, they are typically unstable and should be handled with appropriate care. Thus, although it is possible to isolate them using silica chromatography, concomitant hydrolysis is often substantial and recrystallization or direct use of crude preparations often provide much better alternatives. Indeed, of the most popular of this class, namely D-glucose, D-galactose, N-acetyl-D-glucosamine and D-mannose, only the latter resists crystallization. As a vivid illustration, the perbenzylated glucosyl bromide is, in fact, so unstable that all but the most rapid TLC analyses of its solutions show only the hydrolysis product tetrabenzylglucose (and consequently care should be taken during monitoring of its reactions).

#### Method 5

Preparation of 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide (acetobromoglucose) [15].



*Notes and discussion.* This procedure is a basic method for the preparation of acetobromoglucose. In common with most methods is the use of HBr in glacial acetic acid; some variants also include acetic anhydride as an added reagent, presumably to reverse any potential deacetylation. As a result of the anomeric effect the  $\alpha$ -bromide is the major product. However,  $\beta$ -bromide formation, which presumably occurs prior to anomerization to afford the  $\alpha$ -anomer, and rapid hydrolysis during isolation are likely to account for the remaining mass balance [16].

#### Materials.

1,2,3,4,6-Penta- <i>O</i> -acetyl- $\alpha$ -D-glucopyranose (49.5 g, 127 mmol)	treat as toxic
33% HBr in glacial acetic acid (87.5 ml)	toxic, highly corrosive
Dichloromethane (100 ml $\times$ 2)	risk of irreversible effects
Ice water (200 ml)	no risk

Sodium hydrogencarbonate (aq., sat., 2 × 200 ml)	harmful
Magnesium sulfate	irritant
Ether for crystallization	highly flammable, may form explosive peroxides, harmful
Petroleum ether (bp 40–60 °C) for crystallization	highly flammable, harmful

#### *Equipment.*

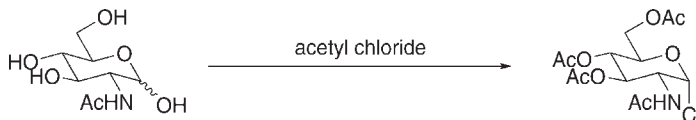
Round-bottomed flask (250 ml) with magnetic stirrer bar  
Magnetic stirrer  
Ice bath  
Conical flask (500 ml)  
Separatory funnel (1000 ml)  
Filtration equipment  
Rotary evaporator

*Special precautions.* Although with this protocol over a short period of time hydrolysis should not be a problem, if prolonged reaction times are employed then dry nitrogen should be introduced. For the same reasons it is also advisable to use oven dried glassware. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. In particular, HBr in acetic acid causes severe burns and must be dispensed in a fumehood with appropriate gloves.

*Procedure.* Add the 1,2,3,4,6-penta-*O*-acetyl- $\alpha$ -D-glucopyranose (49.5 g, 127 mmol) portionwise (~5 g at a time) to a stirred solution of HBr (33%) in glacial acetic acid (87.5 ml) at 0 °C. After all the sugar has been added, allow the reaction mixture to warm to room temperature. After 45 min, TLC analysis (petroleum ether 40/60:ethyl acetate, 1:1) indicates formation of product ( $R_f$  0.5) and consumption of starting material ( $R_f$  0.3). Quench the reaction with ice water (200 ml) and then extract the acetobromoglucose with DCM (2 × 200 ml). Wash the combined organic extracts with a solution of NaHCO<sub>3</sub> (aq., sat., 2 × 200 ml), dry with MgSO<sub>4</sub>, filter and then concentrate *in vacuo*. Crystallize the residue from ether/petrol to afford 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide (46.7 g, 89%) as a white crystalline solid, mp 86–88 °C (ether/petrol);  $[\alpha]_D^{22} = +201$  (*c* 0.5 in CHCl<sub>3</sub>);  $\delta_H$  (200 MHz, CDCl<sub>3</sub>) 2.05, 2.08, 2.12, 2.12 (12H, 4 × s, 4 × COCH<sub>3</sub>), 4.10–4.26 (1H, m, H-6), 4.31–4.38 (2H, m, H-5, H-6'), 4.85, (1H, dd,  $J_{1,2} = 4.0$  Hz,  $J_{2,3} = 10.0$  Hz, H-2), 5.17 (1H, at,  $J = 9.7$  Hz, H-4), 5.57 (1H, at,  $J = 9.7$  Hz, H-3), 6.62 (1H, d, H-1).

*Method 6*

Preparation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl chloride.



*Notes and discussion.* In this procedure, a source of HCl is generated *in situ* through reaction of acetyl chloride with free *N*-acetyl D-glucosamine. This also results in concomitant peracetylation; the peracetate then reacts as expected, with loss of anomeric acetate and, once again as a result of the anomeric effect, the  $\alpha$ -chloride is the major product. If difficulty is experienced with crystallization then purification can be effected by flash chromatography (petroleum ether 40/60:ethyl acetate, 1:2) but yields are typically lower in this case due to partial hydrolysis ( $\sim 60\%$ ).

*Materials.*

2-Acetamido-2-deoxy- $\alpha$ -D-glucopyranose (14.98 g, 67.8 mmol)	treat as toxic
Acetyl chloride (30 ml, 420 mmol)	toxic, highly corrosive
Dichloromethane (150 ml)	risk of irreversible effects
Ice water (100 ml)	no risk
Sodium hydrogencarbonate (aq., sat., 100 ml)	harmful
Magnesium sulfate	irritant
Ether for crystallization (160 + 100 ml)	highly flammable, may form explosive peroxides, harmful

*Equipment.*

Round-bottomed flask (100 ml) fitted with Liebig condenser and magnetic stirrer bar  
 Magnetic stirrer  
 Heat source  
 Source of nitrogen atmosphere  
 Conical flask (250 ml)  
 Separatory funnel (500 ml)

Filtration equipment

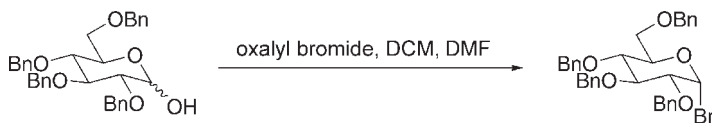
Rotary evaporator

*Special precautions.* To avoid hydrolysis, it is advisable to use oven-dried glassware. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. In particular, acetyl chloride causes severe burns and must be dispensed in a fumehood with appropriate gloves.

*Procedure.* Add the acetyl chloride (30 ml, 420 mmol) by dripping it over the course of 1–2 min down the side of the Liebig-condenser onto 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranose (14.98 g, 67.8 mmol) with stirring. The resulting solution will boil spontaneously for 2 min and then should be refluxed for a further 5 min. Allow the solution to cool to room temperature and stir under nitrogen for a further 15 h. TLC analysis (ethyl acetate/methanol, 9:1) will indicate the formation of a major product ( $R_f$  0.5) and consumption of starting material ( $R_f$  0). Dilute the reaction with DCM (150 ml) and wash with ice-water (100 ml) and then with ice-cold sodium bicarbonate solution (aq., sat., 100 ml). Dry the organic layer with  $MgSO_4$ , filter and concentrate to about 25 ml *in vacuo*. Add dry ether (160 ml) in one portion. Upon standing crystals will form. After 24 h collect the crystals, and wash with dry ether (100 ml) to afford 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl chloride (18.57 g, 75%) as a white crystalline solid, mp 123–125 °C [ $\alpha$ ]<sub>D</sub><sup>23</sup> = +119.4 (c, 1.0 in  $CHCl_3$ );  $\delta_H$  (400 MHz,  $CDCl_3$ ) 1.98, 2.03, 2.04, 2.09 (12H, 4  $\times$  s,  $CH_3$ ), 4.12 (1H, dd,  $J_{5,6} = 2.5$  Hz,  $J_{6,6'} = 13.0$  Hz, H-6), 4.22–4.30 (2H, m, H-5, H-6'), 5.53 (1H, ddd,  $J_{1,2} = 3.7$  Hz,  $J_{2,3} = 10.0$  Hz,  $J_{2,NH} = 8.5$  Hz, H-2), 5.20 (1H, pt,  $J = 10.0$  Hz, H-4), 5.32 (1H, pt, H-3), 5.92 (1H, d,  $J_{2,NH} = 8.5$  Hz, NH), 6.18 (1H, d,  $J_{1,2} = 3.7$  Hz, H-1);  $\delta_C$  (100.6 MHz  $CDCl_3$ ) 20.5, 20.6, 20.9, 23.0 (4  $\times$  s, 4  $\times$   $CH_3$ ), 53.4 (d, C-2), 58.7 (t, C-6), 65.8 (d, C-4), 70.1 (d, C-3), 70.8 (d, C-5), 90.6 (d, C-1), 169.1, 170.1, 170.5, 171.4 (4  $\times$  s, C=O).

#### Method 7

Preparation of 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl bromide [17].



*Notes and discussion.* This procedure is an oxalyl bromide-mediated bromination of the free anomeric hydroxyl of tetra-*O*-benzylglucose. Presumably, an anomeric bromooxalate ester is a primary intermediate in the process, which is then displaced by bromide ion to yield the anomeric bromide. As such the oxalyl bromide is also acting as a source of *in situ* generated HBr. Once again the  $\alpha$ -bromide is the major,

in fact the only product, the  $\beta$ -bromide is presumably all anomerized to afford the  $\alpha$ -anomer. This bromide is highly unstable to silica: attempts to purify by flash chromatography result in low yields of bromide ( $\sim 20\%$ ). As mentioned above, care must therefore also be taken when running and interpreting TLC plates of solutions containing the bromide.

*Materials.*

2,3,4,6-Tetra- <i>O</i> -benzyl-D-glucopyranose (6.1 g, 11 mmol)	treat as toxic
Dried dichloromethane (40 ml)	harmful, flammable
Dried <i>N,N</i> -dimethylformamide (2.5 ml)	flammable, irritant, danger of serious side effects
Oxalyl bromide solution in DCM (12 ml, 2 M, 24 mmol)	harmful, corrosive
Ice water (30 ml)	no risk
Dichloromethane (3 $\times$ 20 ml)	risk of irreversible effects
Brine (40 ml)	no risk
Magnesium sulfate	irritant

*Equipment.*

Round-bottomed flask (100 ml) fitted with inert gas atmosphere and magnetic stirrer bar  
Magnetic stirrer  
Syringe (20 ml) and needle  
Ice bath  
Conical flask (100 ml)  
Separatory funnel (250 ml)  
Filtration equipment  
Rotary evaporator

*Special precautions.* To avoid hydrolysis, it is advisable to use oven-dried glassware. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. In particular, oxalyl bromide is very corrosive and must be dispensed in a fumehood with care and appropriate protection.

*Procedure.* Dissolve 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (6.1 g, 11 mmol) in anhydrous DCM (40 ml) and anhydrous DMF (2.5 ml) under argon and cool



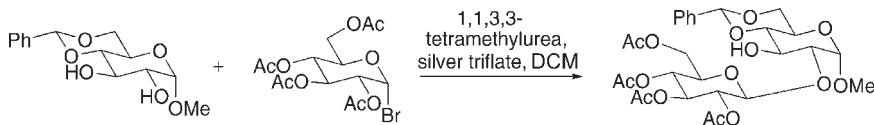
the resulting solution to 0 °C. Add oxalyl bromide (12 ml, 2 M in DCM, 24 mmol) dropwise with stirring over a 5 min period. Care should be taken as there is a rapid evolution of gas. After addition is complete allow the reaction solution to warm to room temperature and stir for a further 40 min. Careful and rapid TLC analysis (petroleum ether/ethyl acetate, 2:1) indicates the formation of a major product ( $R_f$  0.7). Cool the reaction solution again to 0 °C and quench by the careful addition of ice cold water (30 ml) over a 5 min period. Extract with DCM (3 × 20 ml) and combine the organic layers. Wash with brine (40 ml), dry with  $MgSO_4$ , filter and concentrate *in vacuo* to afford 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl bromide (7.07 g, 99%) as a crude oil;  $^1H$  NMR (400 MHz,  $CDCl_3$ ) 3.57 (1H, dd,  $J_{1,2} = 3.5$  Hz,  $J_{2,3} = 9.1$  Hz, H2), 3.68 (1H, dd,  $J_{5,6} = 2.1$  Hz,  $J_{6,6'} = 11.0$  Hz, H-6), 3.79–3.84 (2H, m, H-4, H-6'), 4.07 (1H, at,  $J = 9.1$  Hz, H-3), 4.07–4.11 (1H, m, H-5), 4.47–4.62 (3H, m,  $PhCH_2$ ), 4.74 (s, 2H,  $PhCH_2$ ), 4.84–4.89 (2H, m,  $PhCH_2$ ), 5.10 (1H, d,  $J = 11.1$  Hz,  $PhCH_2$ ), 6.46 (1H, d, H-1), 7.15–7.41 (20H, m, Ar-H).

### 3.3.2 Use of glycosyl bromides and chlorides as glycosyl donors

Glycosyl halides have a long history as donors in glycosylation reactions. The classic glycosylation reaction by Königs and Knorr employed glycosyl bromides [1] and these compounds, as well as glycosyl chlorides, have been used extensively in conjunction with heavy metal salts as halophilic activators, typically silver salts that render the loss of chloride or bromide from the haloether moiety effectively irreversible. The major potential disadvantages are the associated inconvenience and dangers associated with heavy metal salts such as mercury(II) salts and the often large excesses of activator (at least 1 equiv. is needed and often, in practice, several) that may be required.

#### Method 8

Königs–Knorr-type glycosylation of methyl 4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside using acetobromoglucose under silver triflate/tetramethylurea promotion [18, 19].



*Notes and discussion.* This method uses Hanessian's mixed silver triflate and tetramethylurea promoter system as a nice, widely applicable variant on the classical Königs–Knorr glycosylation strategy. Note that the acceptor here possesses two hydroxyl groups and yet good regioselectivity is observed for the less hindered OH-2 hydroxyl group: the mass balance is unreacted acceptor rather than the O-3 regioisomer. This system has been shown to be equally effective in the

glycosylation of e.g. diacetonegalactose or methyl 2,3,6-tri-*O*-benzoyl- $\alpha$ -D-galactopyranoside [18].

*Materials.*

Acetobromoglucose (0.411 g, 1 mmol)	irritant
Methyl 4,6- <i>O</i> -benzylidene- $\alpha$ -D-glucopyranoside (0.564 g, 2 mmol)	treat as toxic
Dried dichloromethane (10 ml)	harmful, flammable
1,1,3,3-Tetramethylurea (0.33 ml, 3 mmol)	irritant, teratogen
Silver triflate (0.567 g, 2.2 mmol)	irritant
Celite <sup>®</sup> for filtration	irritant
Dichloromethane (20 ml)	risk of irreversible effects
Sodium hydrogencarbonate solution (aq., sat., 20 ml)	no risk
Magnesium sulfate	irritant
Toluene for chromatography	highly flammable, harmful
Ethyl acetate for chromatography	highly flammable, irritant

*Equipment.*

Round-bottomed flask (50 ml) fitted with inert gas atmosphere, magnetic stirrer bar and foil to exclude light  
 Magnetic stirrer  
 Syringe (1 ml) and needle  
 Ice bath  
 Conical flask (100 ml)  
 Separatory funnel (100 ml)  
 Filtration equipment  
 Rotary evaporator

*Special precautions.* To avoid hydrolysis, it is advisable to use oven-dried glassware. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. Note that the flash chromatography step can be omitted in favour of direct crystallization using MeOH, however, yields are typically lower.

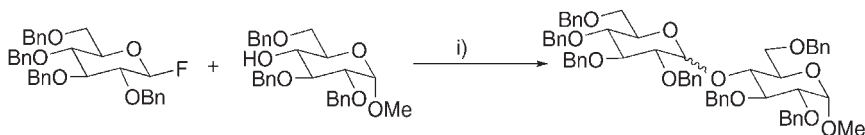
*Procedure.* Dissolve the acetobromoglucose (0.411 g, 1 mmol) in anhydrous DCM (10 ml) under nitrogen and cool to 0 °C. Add the methyl 4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside (0.564 g, 2 mmol), then 1,1,3,3-tetramethylurea (0.33 ml,

3 mmol) and finally the silver triflate (0.567 g, 2.2 mmol) and stir under nitrogen at 0 °C while excluding light from the reaction mixture. After 4 h, filter through Celite<sup>®</sup> and wash the residue with DCM (20 ml). Wash the combined filtrate with sodium hydrogencarbonate solution (aq., sat., 20 ml), dry with magnesium sulfate, filter and remove the solvent. Purify the residue by flash chromatography (toluene/EtOAc, 4:1) to give methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)- $\alpha$ -D-glucopyranoside a colourless oil (0.345 g, 56%) that crystallizes when treated with methanol: mp 226 °C;  $[\alpha]_D = +43$  (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 4.80 (d, 1H,  $J_{1,2} = 3.4$  Hz, H-1), 4.78 (d, 1H,  $J_{1',2'} = 7.8$  Hz, H-1').

### 3.3.3 Preparation of glycosyl fluorides

Glycosyl fluorides have been known for a long while, but were found to be inert to the activation conditions used for the other glycosyl halides and consequently until recently they have been relatively neglected as glycosyl donors. However, in 1981, Mukaiyama found that when a glycosyl fluoride was treated with a mixture of the Lewis acid tin(II) chloride and silver perchlorate, it was activated as a donor [20]. He exemplified this methodology by the synthesis of various glucosides (Scheme 3.1). Subsequent to this breakthrough, many more Lewis acid-based activators have been developed. This new found source of potentially orthogonal glycosylation activity, coupled with the inherent unreactivity of glycosyl fluorides, which bestows stability during many protecting group manipulations, has led to their widespread use as donors (see also Chapter 7).

Glycosyl fluorides are particularly stable during reactions that require basic or neutral conditions. Examples of these include basic ester hydrolysis, to remove acyl protecting groups, and the formation of ether protecting groups using a strong base such as sodium hydride and an alkyl halide. However, glycosyl fluorides are reported to be activated under acidic conditions [21], so care must be taken to avoid any protecting group manipulations that require acidic conditions. Nevertheless, the relative stability of glycosyl fluorides compared to other glycosyl halides, or to trichloroacetimidates, means that they may be introduced into the molecule early in the synthesis. This inherently greater degree of flexibility means that no one standard method for the introduction of fluoride to the anomeric position of a carbohydrate exists and a number are illustrated here.



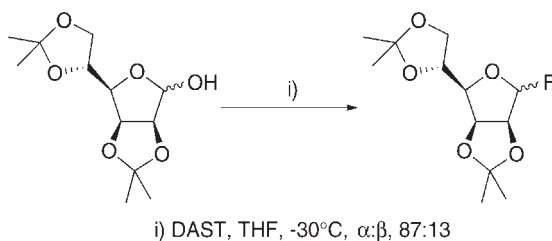
**Scheme 3.1** Mukaiyama's activation of glycosyl fluorides as donors. (i) SnCl<sub>2</sub>, AgClO<sub>4</sub>, –15 °C, ether, 91%,  $\alpha/\beta$  4:1.

Classically, glycosyl fluorides were made by the action of a combination of hydrogen fluoride (HF) and pyridine [22] on an anomeric acetate. However, this rather harsh method is somewhat intolerant of acid-sensitive functionality in the molecule, and furthermore, HF is highly corrosive and methods involving its use have proved unattractive to many researchers.

Diethylaminosulfur trifluoride (DAST) was reported [23, 24] as a highly efficient method for the conversion of the anomeric hemiacetal functionality of a reducing carbohydrate to a glycosyl fluoride. Direct treatment of a thioglycoside with a combination of DAST and *N*-bromosuccinimide (NBS) provides an alternative route to glycosyl fluorides [25]. This is particularly useful, as thioglycosides are stable to acidic protecting group manipulations and therefore this method provides a route to glycosyl fluorides bearing acid-sensitive protection. A recent report details the stereoselective formation of a single 1,2-*trans* anomer of glycosyl fluorides, in both *D*-*gluco* and *D*-*manno* series, by direct treatment of a 1,2-orthoester with DAST [26]. In addition to providing good selectivity, this seems to be the method of choice when it is necessary to distinguish *O*-2 by differential protection of the other hydroxyls. An alternative stereospecific route to the formation of  $\beta$ -*D*-*gluco* fluorides has been reported in two steps from the *D*-glucals. Hereby, epoxidation is followed by ring-opening by tetrabutyl ammonium fluoride (TBAF), to give the  $\beta$ -glucosyl fluoride as the sole 1,2-*trans* anomer [27]. Glycosyl fluorides may be stored for many months at low temperatures, but will often decompose readily in a few days on the open bench. The  $\beta$ -anomers, in particular, are less stable than their  $\alpha$ -counterparts, so appropriate care should be taken to store these compounds in the freezer until needed.

#### Method 9

Formation of a glycosyl fluoride from a hemiacetal: fluorination of 2,3:5,6-di-*O*-isopropylidene- $\alpha$ -*D*-mannofuranose by treatment with DAST [24].



*Notes and discussion.* This procedure is used to form a glycosyl fluoride from a hemiacetal and as such is the original and most straightforward use of DAST in the synthesis of glycosyl fluorides. The product is an anomeric mixture, but there appears to be some solvent dependence of the stereochemical outcome of the reaction, with more polar solvents giving a greater degree of selectivity.

*Materials.*

2,3:5,6-Di- <i>O</i> -isopropylidene- $\alpha$ -D-mannofuranose (1.2 g, 4.5 mmol)	irritant
DAST (diethylaminosulfur trifluoride) (0.66 ml, 5.4 mmol)	toxic, corrosive, reacts violently with water
Anhydrous tetrahydrofuran (12 ml)	irritant, flammable
Chloroform (100 ml)	suspected carcinogen
Distilled water (100 ml)	no risk
Magnesium sulfate	irritant
Ethyl acetate for chromatography	highly flammable, irritant
Petroleum ether (bp 40–60 °C) for chromatography	highly flammable, harmful

*Equipment.*

Bunsen burner

Round-bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  $\times$  2

Cannula

Magnetic stirrer

Cooling bath for  $-30\text{ }^{\circ}\text{C}$

Source of dry argon (or nitrogen) as inert gas

Microsyringe (1000  $\mu\text{l}$ ) and needle

Separatory funnel (500 ml)

Rotary evaporator

Flash chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under an inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. DAST is extremely moisture-sensitive, so care must be taken when handling it. Additionally, it should be stored in the fridge to avoid decomposition. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round bottomed flask with a magnetic stirrer bar and a rubber septum and flush with argon. A balloon of argon can be used to maintain the inert

atmosphere during the reaction. Add the 2,3:5,6-di-*O*-isopropylidene- $\alpha$ -D-mannofuranose (1.2 g, 4.5 mmol) by dissolving it in the anhydrous tetrahydrofuran (12 ml) under argon and add it to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Cool the reaction vessel to  $-30\text{ }^{\circ}\text{C}$  in the cooling bath. Using a microsyringe add DAST (0.66 ml, 5.4 mmol), which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. Immediately remove the reaction vessel from the cooling bath. After 20 min the reaction will be complete, as indicated by TLC analysis (petroleum ether/ethyl acetate 3:1): the  $\alpha$ -product should be visible as a major product spot, with the  $\beta$ -product as a minor, slower-running product spot. The reaction is worked-up by the addition of chloroform (100 ml). The resulting solution should be transferred into a separatory funnel and washed with distilled water (100 ml). Dry the organic layer with magnesium sulfate, filter and remove the solvent *in vacuo* to give the crude product. Purify by flash chromatography (ether/ethyl acetate 3:1). The chromatographically more mobile  $\alpha$ -fluoride can be isolated as a colourless syrup (87% yield).  $[\alpha]_{\text{D}}^{25} = -14.2$  (*c* 0.8,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 5.69 (d,  $J = 59.5$  Hz, H-1, 1H),  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 113.7 (d,  $J = 221.5$  Hz, C-1). The less mobile  $\beta$ -fluoride, which can be recrystallised from petroleum ether, is eluted next in 13% yield.  $[\alpha]_{\text{D}}^{25} = -7.7$  (*c* 1.1,  $\text{CHCl}_3$ ); mp  $115\text{--}116\text{ }^{\circ}\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 5.51 (dd,  $J = 66.5, 3.7$  Hz, H-1, 1H),  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 107.5 (d,  $J = 235.6$  Hz, C-1); Anal. Calc. for  $\text{C}_{12}\text{H}_{19}\text{FO}_5$ : C, 55.0; H, 7.3; F, 7.2. Found: C, 55.3; H, 7.3; F, 7.0%.

### Method 10

Formation of a glycosyl fluoride with concomitant differential protection of *O*-2: ring-opening of the orthoester, 3,4,6-tri-*O*-benzyl-1,2-*O*-(*exo*-methoxyethylidene)- $\beta$ -D-mannopyranose with DAST [26].



**Notes and discussion.** This procedure is used for the stereoselective formation of a 1,2-*trans* configured fluoride. The use of the orthoester precursor allows differentiation of the *O*-2, which is a strategic manipulation that is often useful in oligosaccharide synthesis. The product also has participatory acetyl protection of *O*-2, which may be used to direct the stereochemistry of its glycosylation reaction. The direct treatment with DAST has the advantage that only a single anomer is formed, compared to the anomeric mixture which results from the two step process of hydrolysis to the hemiacetal followed by DAST treatment.

*Materials.*

3,4,6-Tri- <i>O</i> -benzyl-1,2- <i>O</i> -( <i>exo</i> -methoxyethylidene)- $\beta$ -D-mannopyranose (16.7 g, 32.8 mmol)	irritant
Diethylaminosulfur trifluoride (DAST, 5.1 ml, 37.7 mmol)	toxic, corrosive, reacts violently with water
Diethyl ether	highly flammable
Anhydrous dichloromethane (80 ml, distilled from CaH <sub>2</sub> )	highly flammable, harmful
Anhydrous magnesium sulfate	irritant
Ethyl acetate for chromatography	highly flammable, irritant
Petroleum ether (bp 40–60 °C) for chromatography	highly flammable, harmful
Triethylamine	corrosive, lachrymator, toxic

*Equipment.*

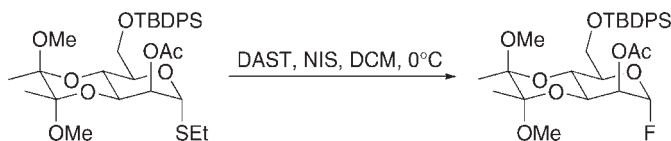
Bunsen burner  
 Round-bottomed flask (250 ml) with rubber septum and magnetic stirrer bar  $\times$  2  
 Cannula  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) as inert gas  
 Syringe (10 ml) and needle  
 Ice bath  
 Separatory funnel (1 l)  
 Filtration equipment  
 Rotary evaporator  
 Flash chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under an inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. DAST is extremely moisture-sensitive, so care must be taken when handling it. Additionally it should be stored in the fridge, to avoid decomposition. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

**Procedure.** Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 3,4,6-tri-*O*-benzyl-1,2-*O*-(*exo*-methoxyethylidene)- $\beta$ -D-mannopyranose (16.7 g, 32.8 mmol) by dissolving it in anhydrous dichloromethane (80 ml) under argon and then adding the resulting solution to the reaction vessel via a cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Place the reaction vessel in the ice bath and allow it to cool. Using a syringe add DAST (5.1 ml, 37.7 mmol), which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. After 7 h the reaction will be complete, as indicated by tlc analysis (petroleum ether/ethyl acetate 3:1): the fluoride should be visible as a major product spot (a more polar intermediate is also formed during the course of the reaction but it is also converted to the fluoride product). Work up the reaction by addition of diethyl ether (200 ml) and transfer the resulting solution into a separatory funnel. Wash with distilled water (200 ml). Dry the organic layers using magnesium sulfate, filter and remove the solvent *in vacuo* to give the crude product. Purify by flash chromatography (petroleum ether 40/60–ethyl acetate 5:1). It is useful to add 1% triethylamine to the eluent in order to minimize decomposition of the product, which is sensitive to acidic silica. The compound can be isolated as colourless syrup (98% yield).  $[\alpha]_D^{23} = +13.3$  (*c* 1.0 in  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.17 (s,  $\text{CH}_3$ , 3H), 3.72 (d,  $J = 10.9$  Hz, H-6, 1H), 3.82 (dd,  $J = 2.7, 10.9$  Hz, H-6', 1H), 3.97–3.99 (m, H-3, H-4, H-5, 3H), 4.49, 4.87 (ABq,  $J = 10.7$  Hz,  $\text{PhCH}_2$ , 2H), 4.52, 4.69 (ABq,  $J = 12.1$  Hz,  $\text{PhCH}_2$ , 2H), 4.57, 4.72 (ABq,  $J = 10.9$  Hz,  $\text{PhCH}_2$ , 2H), 5.48 (br s, H-2, 1H), 5.62 (dd,  $J = 1.9, 49.1$  Hz, H-1, 1H), 7.15–7.38 (m, Ar-H, 15H); Anal. Calc. for  $\text{C}_{29}\text{H}_{31}\text{O}_6\text{F}$ : C, 70.43; H, 6.32. Found: C, 70.27; H, 6.50%.

### Method 11

Formation of a glycosyl fluoride from a thioglycoside: treatment of 2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*,4-*O*-(2',3'-dimethoxybutan-2',3'-diyl)-1-thio- $\alpha$ -D-mannopyranoside with a combination of DAST and *N*-iodosuccinimide [28].



**Notes and discussion.** This procedure shows another versatile method for the interconversion of anomeric functionality. The glycosyl fluoride formed in this case bears a butane-1,2-diacetal protecting group, which is introduced under acidic conditions. The fluoride may be unstable to these conditions, whereas protection of a thioglycoside is possible. The synthetic strategy therefore effectively involves the use of a thioglycoside as a 'masked' glycosyl fluoride. The procedure described



here employs the more reactive *N*-iodosuccinimide, as compared to *N*-bromosuccinimide originally reported for such a conversion [25].

*Materials.*

2- <i>O</i> -Acetyl-6- <i>O</i> - <i>tert</i> -butyldiphenylsilyl-3- <i>O</i> ,4- <i>O</i> -(2',3'-dimethoxybutan-2',3'-diyl)-1-thio- $\alpha$ -D-mannopyranoside (7.12 g, 11.5 mmol)	irritant
Diethylaminosulfur trifluoride (DAST, 3.0 ml, 23.1 mmol)	toxic, corrosive, reacts violently with water.
<i>N</i> -Iodosuccinimide (NIS, 3.38 g, 15.0 mmol)	severe irritant
Anhydrous dichloromethane (125 ml, distilled from CaH <sub>2</sub> )	highly flammable, harmful
Sodium bicarbonate/sodium thiosulfate solution (1:1 aq. v/v, 150 ml)	harmful
Anhydrous magnesium sulfate for drying	irritant
Ethyl acetate for chromatography	highly flammable, irritant
Petroleum ether (bp 40–60 °C) for chromatography	highly flammable, harmful

*Equipment.*

Bunsen burner  
 Round-bottomed flask (500 ml) with a rubber septum plus magnetic stirrer bar  $\times$  2  
 Cannula  
 Magnetic stirrer  
 Ice bath  
 Source of dry argon (or nitrogen) as inert gas  
 Syringe (5 ml) and needle  
 Separatory funnel (500 ml)  
 Filtration equipment  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under an inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. DAST

is extremely moisture-sensitive, so care must be taken when handling it. Additionally, it should be stored in the fridge to avoid decomposition. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush with argon, then flame dry and allow to cool, while maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*,4-*O*-(2',3'-dimethoxybutan-2',3'-diyl)-1-thio- $\alpha$ -D-mannopyranoside (7.12 g, 11.5 mmol) to the flask by dissolving it in anhydrous dichloromethane (125 ml) under argon and then adding the resulting solution to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Cool the resulting reaction solution to 0 °C by immersing the reaction vessel in an ice bath. Use a syringe to add DAST (3.0 ml, 23.1 mmol), which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. After 20 min, add the NIS (3.38 g, 15.0 mmol) and remove the reaction vessel from the ice-bath. You will see the formation of a deep purple colour. After a further 2 h and 30 min, during which time the mixture is allowed to warm to room temperature, the reaction will be complete. The reaction is worked-up by transferring into a separatory funnel and washing with saturated aqueous sodium bicarbonate and 10% aqueous sodium thiosulfate solution (1:1 v/v, 150 ml). Dry the organic layers using magnesium sulfate, filter and remove the solvent *in vacuo* to give the crude product. Purify by column chromatography (petroleum ether 40/60–diethyl ether, 12:1). The compound can be isolated as a foam in 88% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.07 (s, SiC(CH<sub>3</sub>)<sub>3</sub>, 9H), 1.30 (s, CH<sub>3</sub> BDA, 3H), 1.31 (s, CH<sub>3</sub> BDA, 3H), 2.16 (s, COCH<sub>3</sub>, 3H), 3.26 (s, OCH<sub>3</sub> BDA, 3H), 3.28 (s, OCH<sub>3</sub> BDA, 3H), 3.90–3.87 (m, H-5, H-6, 2H), 4.03 (dd, *J* = 11.6, 2.6 Hz, H-6', 1H), 4.18 (dd, *J* = 10.5, 2.8 Hz, H-3, 1H), 4.41 (t, *J* = 10.3, H-4, 1H), 5.19 (s, H-2, 1H), 5.60 (d, *J* = 49.1 Hz, H-1, 1H), 7.71–7.35 (m, Ar-H, 10H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 17.7, 17.8 (2 × CH<sub>3</sub> BDA), 19.5 (SiC(CH<sub>3</sub>)<sub>3</sub>), 21.0 (COCH<sub>3</sub>), 26.8 (SiC(CH<sub>3</sub>)<sub>3</sub>), 48.1, 48.2 (2 × OCH<sub>3</sub> BDA), 61.1 (C-6), 61.6 (C-4), 65.3 (C-3), 69.0 (d, *J* = 37.8 Hz, C-2), 73.9 (C-5), 99.8, 100.3 (2 × acetal C BDA), 105.4 (d, *J* = 223 Hz, C-1), 127.6, 129.6, 129.7 (Ar-C), 132.9, 134.1 (Ar-C *ipso*) 135.4, 136.0 (Ar-C), 170.4 (CO); Anal. Calc. for C<sub>30</sub>H<sub>41</sub>FO<sub>8</sub>Si: C, 62.48; H, 7.17. Found: C, 62.3; H, 7.2%.

### 3.3.4 Use of glycosyl fluorides as glycosyl donors

In typical procedures for glycosyl fluoride glycosylation reactions, the glycosyl fluoride is mixed with an aglycon alcohol and a Lewis acid promoter. As noted above, Mukaiyama's original disclosure described the use of a combination of tin(II) chloride and silver perchlorate as the activator [20], but since then, many other

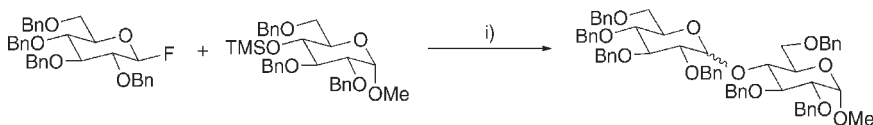
activators have been reported to be effective in glycoside synthesis. A high yielding method involving metallocenes in combination with silver salts has been reported by Suzuki and co-workers [29]. It was found that the use of a 1:2 ratio of bis-cyclopentadienyl hafnocene dichloride and silver perchlorate was the optimum reaction condition. This led them to postulate that, in fact, the reactive species is bis-cyclopentadienyl hafnium diperchlorate, and that the driving force for the reaction is the formation of the strong hafnium–fluorine bond.

Many of the promoters used are either toxic or expensive, or both. Catalytic activation possesses a distinct advantage then, in that only a relatively small amount of the promoter is required. Various catalytic promoters have been reported. These include boron trifluoride etherate [30], titanium tetrafluoride [31] and even various Brønsted acids [21].

A useful twist on this methodology was introduced by Noyori, who, in place of aglycon alcohol, used its trimethylsilyl ether derivative, thus allowing glycosylation with a catalytic amount of a silicon-based Lewis acid, either silicon tetrafluoride, or trimethylsilyl trifluoromethanesulfonate (TMSOTf) (Scheme 3.2) [32]. In this method, a strong silicon fluorine bond is formed, generating at the same time a glycosyl cation or cation-like intermediate. This is captured by the nucleophilic TMS-protected aglycon oxygen, forming the glycosidic bond. This process occurs with concomitant desilylation, thus regenerating the TMSOTf catalyst.

The stereoselectivity of a glycosylation reaction is crucial to its utility in synthesis. Therefore, every effort must be taken to plan carefully in order to achieve the desired stereochemical outcome. The selectivity of any given reaction is influenced by a number of factors: the glycosyl donor, the glycosyl acceptor, the choice of promoter and the solvent. With so many variables, it may seem a daunting task to predict the stereochemistry of a glycosylation reaction. Nevertheless, certain general guidelines are useful. If the donor bears a participatory protection of *O*-2, selectivity in favour of the 1,2-*trans* glycoside is seen. It has also been seen that, in general, the use of acetonitrile as solvent favours the formation of  $\beta$ -glycosides, whereas when the reaction is carried out in diethyl ether, a product ratio in favour of the  $\alpha$  anomer is seen.

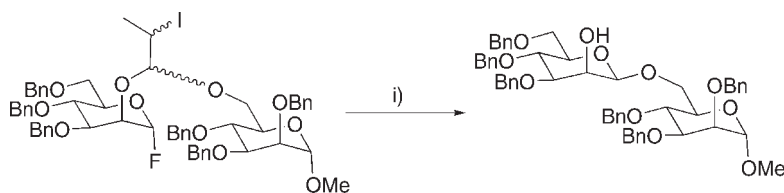
In the case of Brønsted acid-mediated glycosyl fluoride donor glycosylations, the effect of counter-ion was also found to be as important as that of solvent [21]. For instance, trifluoromethanesulfonic acid favoured the formation of the  $\alpha$ -glycoside, whereas bis-trifluoromethanesulfonamide favoured  $\beta$ . Interestingly, this phenomenon was emphasised by the demonstration of matched and mismatched selectivities



**Scheme 3.2** Noyori's catalytic activation of glycosyl fluorides using TMS protected acceptors. (i)  $\text{SiF}_4$  (20 mol%), MeCN, 0 °C, 90%,  $\alpha/\beta$  9:91.

in different solvents. Thus, when a glycosylation reaction was carried out using trifluoromethanesulfonic acid ( $\alpha$ -promotor) in diethyl ether ( $\alpha$ -solvent), good  $\alpha$ -selectivity was seen ( $\alpha/\beta$ , 88:12). In a mixed solvent of trifluoromethylbenzene (BTF) and pivalonitrile ( $t$ BuCN) ( $\beta$ -favouring solvent), the selectivity was lowered to 47:53. Conversely, an analogous reaction using the  $\beta$ -favouring promotor bis-trifluoromethanesulfonamide gave little selectivity in diethyl ether ( $\alpha$ -solvent) ( $\alpha/\beta$ , 49:51) whereas in BTF- $t$ BuCN ( $\beta$ -solvent), a good selectivity of 9:91 was observed. Thus, it may be seen that in the absence of any stereochemically defining group, such as a participatory neighbouring group, the choice of activator and solvent, and indeed their combination, is of great importance in the use of glycosyl fluorides.

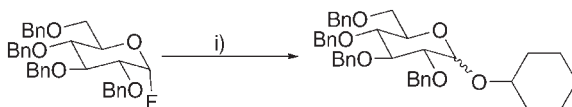
The principle of intramolecular aglycon delivery has been applied to glycosyl fluorides, using a *para*-methoxybenzyl ether [33] and more recently an allyl ether-derived tether [34]. In these processes, the aglycon is temporarily tethered to a modified protecting group at *O*-2 of the donor prior to glycosylation. This means that upon subsequent activation of the glycosyl fluoride, the aglycon may only be delivered from the face to which it is tethered, giving the 1,2-*cis* glycoside as the sole glycosylated product (Scheme 3.3).



**Scheme 3.3** Intramolecular aglycon delivery using glycosyl fluorides. (i)  $\text{SnCl}_4$ ,  $\text{AgClO}_4$ , DTBMP,  $50^\circ\text{C}$ , DCE, 75%.

### Method 12

Glycosylation using a glycosyl fluoride donor: activation of 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl fluoride with a combination of bis-cyclopentadienyl hafnium dichloride ( $\text{Cp}_2\text{HfCl}_2$ ) and silver perchlorate ( $\text{AgClO}_4$ ).



i)  $\text{Cp}_2\text{HfCl}_2$ ,  $\text{AgClO}_4$ , DCM,  $-50^\circ\text{C}$ ,  $\alpha/\beta$ , 11:1

**Notes and discussion.** This procedure demonstrates the popular procedure for metallocene-mediated activation of glycosyl fluorides [29]. Illustrated here is Suzuki's modified procedure, whereby a molar ratio of ( $\text{Cp}_2\text{HfCl}_2/\text{AgClO}_4$ , 1:2) provides impressively enhanced reactivity over the originally reported 1:1 ratio.

In addition, pre-mixing of the  $\text{Cp}_2\text{HfCl}_2$  and  $\text{AgClO}_4$  at room temperature prior to cooling and then addition of the alcohol acceptor and glycosyl fluoride donor is beneficial. This precaution ensures the formation of the activator complex *in situ*, which may not occur to the desired extent if all the reactants are mixed at low temperature.

*Materials.*

2,3,4,6-tetra- <i>O</i> -benzyl- $\alpha$ -D-glucopyranosyl fluoride (61 mg, 113 $\mu\text{mol}$ )	irritant
Cyclohexanol (23 mg, 226 $\mu\text{mol}$ )	irritant
Bis-cyclopentadienyl hafnium dichloride ( $\text{Cp}_2\text{HfCl}_2$ ) (43 mg, 113 $\mu\text{mol}$ )	irritant
Silver perchlorate ( $\text{AgClO}_4$ ) (47 mg, 227 $\mu\text{mol}$ )	explosive
Anhydrous dichloromethane (2 ml, distilled from $\text{CaH}_2$ )	highly flammable, harmful
4 Å powdered molecular sieves	irritant
Dichloromethane (30 ml)	highly flammable, harmful
Sodium bicarbonate solution (aq., sat., 30 ml)	harmful
Anhydrous magnesium sulfate for drying	irritant
Ethyl acetate for chromatography	highly flammable, irritant
Petroleum ether (bp 40–60 °C) for chromatography	highly flammable, harmful

*Equipment.*

Bunsen burner  
 Round-bottomed flask (10 ml) with a rubber septum plus magnetic stirrer bar  $\times$  2  
 Cannula  
 Magnetic stirrer  
 Cooling bath for  $-50^\circ\text{C}$   
 Source of dry argon (or nitrogen) as inert gas  
 Syringe (5 ml) and needle  
 Separatory funnel (100 ml)  
 Filtration equipment  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere.

Additionally, once the glassware has been assembled and placed under an inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow it to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add  $\text{Cp}_2\text{HfCl}_2$  (43 mg, 113  $\mu\text{mol}$ ),  $\text{AgClO}_4$  (47 mg, 227  $\mu\text{mol}$ ), 4 Å powdered molecular sieves and anhydrous dichloromethane (0.5 ml) to the flask. Stir the mixture at room temperature for 10 min. Add cyclohexanol (23 mg, 226  $\mu\text{mol}$ ) to the flask by dissolving it in anhydrous dichloromethane (0.5 ml) under argon and adding the resulting solution to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Cool the solution to  $-50^\circ\text{C}$  by immersing the reaction vessel in a cooling bath. Add 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl fluoride (61 mg, 113  $\mu\text{mol}$ ) to the flask by dissolving it in anhydrous dichloromethane (1 ml) under argon and adding the resulting solution to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). After 1 h, the reaction will be complete. The reaction is worked-up by diluting with dichloromethane (30 ml), transferring into a separatory funnel and washing with saturated aqueous sodium bicarbonate solution (30 ml). Dry the organic layers using magnesium sulfate, filter and remove the solvent *in vacuo* to give the crude product. Purify by flash chromatography (petroleum ether 40/60:ethyl acetate 3:1). The compound can be isolated in 93% yield, ( $\alpha/\beta$ , 11:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) for  $\alpha$ -anomer 1.15–2.10 (m, cyclohexyl  $\text{CH}_2$ , 10H), 3.59 (m, cyclohexyl CH, 1H), 3.60 (dd,  $J = 9.5, 4.0$  Hz, H-2, 1H), 3.68 (t,  $J = 9.5$  Hz, H-4, 1H), 3.66 (m, H-6, 1H), 3.78 (dd,  $J = 10.4, 3.7$  Hz, H-6', 1H), 3.93 (m, H-5, 1H), 4.05 (t,  $J = 9.5$  Hz, H-3, 1H), 4.50, 4.66 (ABq,  $J = 12.0$  Hz,  $\text{PhCH}_2$ , 2H), 4.52, 4.87 (ABq,  $J = 10.7$  Hz,  $\text{PhCH}_2$ , 2H), 4.69, 4.79 (ABq,  $J = 12.0$  Hz,  $\text{PhCH}_2$ , 2H), 4.85, 5.04 (ABq,  $J = 12.0$  Hz,  $\text{PhCH}_2$ , 2H), 5.00 (d,  $J = 4.0$  Hz, H-1, 1H), 7.13–7.44 (m, Ar-H, 20H)  $\beta$ -anomer 4.50 (d,  $J = 8.1$  Hz, H-1, 1H).

### 3.4 PHENYL SELENIDES

The use of selenium in carbohydrate chemistry dates as far back as 1917 with the synthesis of selenoisotrehalose [35]. However, it was not until 1991 that selenoglycosides were first used as glycosyl donors [36]. It should be noted that while a wide variety of selenoglycosides are reported in the literature [37] their use as glycosyl donors has to date been confined almost exclusively to phenyl

selenoglycosides. Consequently, the focus of this section will be specifically the generation and activation of phenyl selenoglycosides.

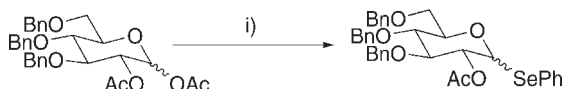
The impetus to investigate the use of phenyl selenoglycosides as glycosyl donors came from increasing interest in the selective activation of glycosyl donors. Pinto demonstrated [36] that it was possible to choose a promoter which selectively activates phenyl selenoglycosides in the presence of thioglycosides, or conversely to selectively activate glycosyl bromides or trichloroacetimidates in the presence of phenyl selenoglycosides. This methodology was expanded by van Boom who showed that the armed/disarmed concept, first proposed by Fraser-Reid [38] and discussed in Chapter 6, which had been shown to apply to thioglycosides, is equally applicable to their seleno analogues [39].

It is a combination of these two approaches—variation of the activating agent and reactivity tuning by variation of the protecting group regime—that have led to the increasing use of phenyl selenoglycosides in various elegant oligosaccharide syntheses [40].

### 3.4.1 Preparation of phenyl selenides

Compounds of selenium are highly toxic and in addition have a tendency, like their sulfur analogues, to have an unpleasant odour. Care is therefore necessary when handling selenium reagents, and it is advisable to avoid the more pungent chemicals where an alternative is available. In particular, selenophenol itself is extremely noxious and should be avoided if possible. As phenyl selenoglycosides are stable to both basic [41] and acidic [40] conditions it is often possible for the functionality to be introduced early in a given synthesis.

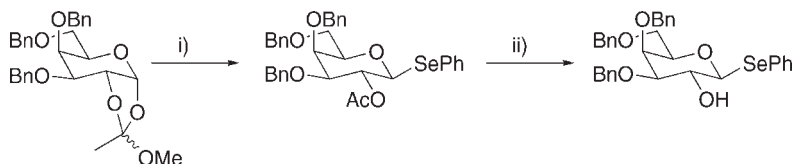
A number of preparative methods have been reported in the literature. It is possible to react a glycoside containing an ester group (usually acetate) at the anomeric position directly with selenophenol [42] in the presence of a Lewis acid (usually  $\text{BF}_3 \cdot \text{OEt}_2$ ) to give the phenyl selenoglycoside, in an analogous manner to that frequently used for the synthesis of thioglycosides (Scheme 3.4). Alternatively phenyltrimethylsilyl selenide in the presence of a catalytic amount of trimethylsilyl triflate has been shown to give the phenyl selenoglycoside when reacted with glucose pentaacetate over 2 days [43].



**Scheme 3.4** Lewis acid catalysed phenyl selenoglycoside formation (i)  $\text{PhSeH}$ ,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , DCM.

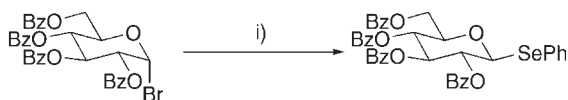
More recently Sinaÿ showed that the reaction of a C-1,2 orthoester with selenophenol in the presence of mercury(II) bromide as a Lewis acid catalyst gave the corresponding selenoglycoside [44]. This leaves an acetate at the *O*-2 position of

the sugar, which can be selectively removed if an appropriate protecting group strategy has been employed, making this the method of choice if selective access to *O*-2 is required (Scheme 3.5).



**Scheme 3.5** Opening of an orthoester to give selective access to *O*-2 (i) PhSeH, HgBr<sub>2</sub>, MeCN, 50 °C, 1 h, 78%; (ii) NaOMe, MeOH, 100%.

A more common route is the generation of the selenophenyl anion followed by displacement of an anomeric halide. The selenophenyl anion can be generated either directly from the selenophenol by the addition of a strong base such as KOH [78] or by reduction of diphenyldiselenide (Scheme 3.6) [44–46]. This reductive methodology is often used as it circumvents the need to handle selenophenol directly (salts of selenophenol are not commercially available) and the glycosyl halide can be added directly to the reaction vessel after reduction of the diselenide. These reactions are generally carried out with a participating group (e.g. acetate) at the *O*-2 position in order to obtain a single stereoisomer, although this is not crucial if the intention is to utilise the phenyl selenoglycoside as a glycosyl donor.



**Scheme 3.6** Stick's phenyl selenoglycoside synthesis (i) PhSeSePh, NaBH<sub>4</sub>, EtOH, 2 h, 88%.

Pinto utilised a similar methodology, employing the reduction of diphenyldiselenide with hypophosphorus acid, followed by reaction of the selenophenol generated with a peracetylated sugar in the presence of a Lewis acid (boron trifluoroetherate) [47]. However, this methodology lacks the simplicity of the above diphenyldiselenide reductions, requiring manipulation of the selenide containing solution before addition of the glycoside.

Other methods of selenoglycoside synthesis include the use of selenostannane in the presence of catalytic Bu<sub>2</sub>Sn(OTf)<sub>2</sub> [48] and the opening by selenophenol of a 1,2-epoxide (Brigl anhydride) [49], however, these methods are less frequently employed.

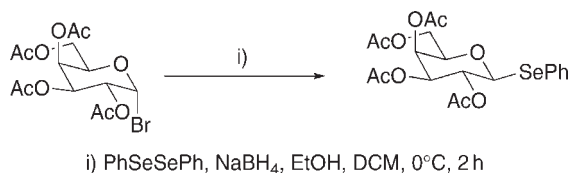
Another useful method for the preparation of phenyl selenoglycosides is the azidoselenation reaction, in which a glycal is reacted with sodium azide, diphenyldiselenide and (diacetoxyiodo)benzene to give a 2-azido-2-deoxy-selenoglycoside. The reaction is believed to proceed through a radical mechanism, and



gives the glycoside with excellent regioselectivity. However, it is common to obtain a mixture of stereoisomers [50]. This is a relatively mild, single step route from a glycal to the corresponding 2-azido glycoside containing an anomeric leaving group. Further manipulations allow rapid access to phenyl2-amino-2-deoxy-1-selenoglycosides (via azide reduction) [51], 2-azido-2-deoxy-glycoses (via hydrolysis) [52] or azido-containing oligosaccharides [53].

### Method 13

Generation of a phenyl selenoglycoside from 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-galactopyranosyl bromide via displacement of the anomeric halide with a selenophenol anion generated by reduction of diphenyldiselenide [44].



*Notes and discussion.* This is probably the most convenient method of forming glycosyl selenides, avoiding, as it does, the need to handle selenophenol. The diphenyl diselenide is a commercially available crystalline yellow solid, which can be easily handled and has only a mildly offensive odour. The glycosyl halide can alternatively be dissolved in CHCl<sub>3</sub>, EtOH or even added directly to the reaction mixture. If the reaction is left for too long, some deacetylation is seen. However, if a longer reaction time is required alternative protecting groups, such as benzoate, may be used [46].

### Materials.

2,3,4,6-Tetra- <i>O</i> -acetyl- $\alpha$ -D-galactopyranosyl bromide (12 g, 29.9 mmol)	irritant
Sodium borohydride (1.43 g, 38 mmol)	burns
Diphenyldiselenide (5.9 g, 19 mmol)	toxic
Anhydrous ethanol (100 ml, distilled from CaH <sub>2</sub> )	highly flammable
Anhydrous dichloromethane (20 ml, distilled from CaH <sub>2</sub> )	highly flammable, harmful
Dichloromethane (200 ml)	risk of irreversible effects
Distilled water (200 ml)	no hazard
Sodium hydroxide (200 ml, 10% solution in water)	causes severe burns

Magnesium sulfate	irritant
Cyclohexane for chromatography	highly flammable, irritant
Ethyl acetate for chromatography	highly flammable, irritant

*Equipment.*

Round-bottomed flask (250 ml) with rubber septum and magnetic stirrer bar  $\times$  2  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Ice bath  
Cannula  
Separatory funnel (500 ml)  
Rotary evaporator  
Flash chromatography equipment

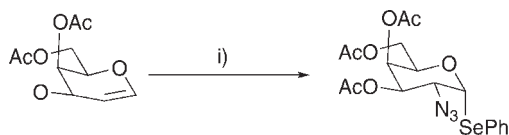
*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions in an inert atmosphere. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. The diphenyldiselenide should be dispensed in fumehood. If a balance is not available within the fumehood then the diphenyldiselenide should be dispensed into a sealable container (e.g. a vial) of known mass before removal for weighing. Additionally a bleach solution should be prepared (sodium hypochlorite solution/water, 1:10. A small amount of sodium hydroxide can be added if a stronger bleaching effect is required) and placed in the fumehood. All used glassware should be placed into this once finished with, to oxidize any trace selenides that may remain. This will also avoid any unnecessary odour. Note also that some of the early column fractions are likely to need similar treatment; these can usually be easily identified by their yellow colouration. However, care should be taken to ensure no compounds that may react violently with the bleach solution are treated in this manner. It is highly recommended that the rotary evaporator used be contained within the fumehood. If this is not possible a bleach solution placed in the solvent trap will help reduce any odour. In either case the rotary evaporator should be thoroughly cleaned after use.

*Procedure.* Once the glassware is dry, equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add diphenyldiselenide (5.9 g, 19 mmol) and anhydrous ethanol (100 ml, distilled from  $\text{CaH}_2$ ) and cool the reaction to 0 °C using an ice bath. The solution will be a yellow colour, due to the diphenyldiselenide. Dissolve the sodium borohydride (1.43 g, 38 mmol) in

ethanol (20 ml, distilled from  $\text{CaH}_2$ ) and cool to  $0^\circ\text{C}$ . Add this borohydride solution to the reaction mixture via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Stir the solution for 15 min at  $0^\circ\text{C}$  (this is to prevent a potentially violent, exothermic, reaction occurring) and then for 20 min at room temperature. At this point the solution should be colourless, as all the diphenyldiselenide has been consumed. If any yellow colouration persists more sodium borohydride should be added until the solution is colourless. Now dissolve the 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-galactopyranosyl bromide (12 g, 29.9 mmol) in anhydrous dichloromethane (20 ml, distilled from  $\text{CaH}_2$ ) and add this to the reaction mixture via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Stir the solution at room temperature for a further 2 h. After this time the reaction will be complete, as indicated by TLC analysis (cyclohexane/ethyl acetate, 3:1). Work up the reaction by allowing it to stir for 15 min in the open air (in order to oxidise any remaining  $\text{PhSeNa}$ ) and then by removing the solvent *in vacuo*. Dissolve the residue in dichloromethane (200 ml) and transfer to a separatory funnel. The solution should be washed with distilled water (200 ml), sodium hydroxide (200 ml, 10% solution in water) and distilled water again (200 ml). Drying the organic layer with magnesium sulfate, filtration and removal of the solvent *in vacuo* affords the crude product which should be purified by flash chromatography (cyclohexane/ethyl acetate 6:1  $\rightarrow$  2:1). The compound is a syrup (93% yield).  $[\alpha]_D^{21} = -2$  ( $c$  0.8,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) 1.97, 2.04, 2.09, 2.10 ( $4 \times s$ ,  $4 \times \text{COCH}_3$ , 12H), 3.87–3.95 (m, H-5, 1H), 4.09 (dd,  $J = 6.1, 11.2$  Hz, H-6, 1H), 4.18 (dd,  $J = 7.2, 11.2$  Hz, H-6, 1H), 4.93 (d,  $J = 10.0$  Hz, H-1, 1H), 5.03 (dd,  $J = 3.1, 9.8$  Hz, H-3, 1H), 5.28 (dd,  $J = 9.8, 10.0$  Hz, H-2, 1H), 5.42 (d,  $J = 3.1$  Hz, H-4, 1H), 7.26–7.66 (m, ArH, 5H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 20.5–20.8 ( $4 \times \text{CH}_3\text{CO}$ ), 61.5, 67.2, 67.9, 71.7, 75.4 (C-2, C-3, C-4, C-5, C-6), 81.6 (C-1), 127.6–134.8 (Ar), 169.4–170.3 ( $4 \times \text{CH}_3\text{CO}$ );  $m/z$  (CI) 507 ( $\text{M} + \text{NH}_4^+$ ).

#### Method 14

Azidophenylselenation of 3,4,6-tri-*O*-acetyl-D-galactal [52].



i) (diacetoxyiodo) benzene,  $\text{NaN}_3$ ,  $\text{PhSeSePh}$ , DCM, 48 h, 92%

*Notes and discussion.* This reaction is believed to proceed via a radical mechanism to regiospecifically afford the anti-Markovnikov product. This procedure is representative of that used for the azidoselenation of many glycals, however in most cases a mixture of isomers at the C-2 position is obtained and in some cases an

anomeric mixture is also observed [52]. It is also possible to obtain the Markovnikov product if PhSeCl is used to initiate the reaction [54].

*Materials.*

3,4,6-Tri- <i>O</i> -acetyl-D-galactal (272 mg, 1 mmol)	irritant
Diphenyldiselenide (187 mg, 0.6 mmol)	toxic
Sodium azide (156 mg, 2.4 mmol)	highly toxic, explosive
Anhydrous dichloromethane (5 ml, distilled from CaH <sub>2</sub> )	irritant, flammable
(Diacetoxyiodo)benzene (451 mg, 1.4 mmol)	assume toxic
Dichloromethane (30 ml)	risk of irreversible effects
Sodium hydrogencarbonate (16 ml, saturated solution in water)	irritant
Distilled water	no risk
Magnesium sulfate	irritant
Hexane for chromatography	flammable, irritant
Diethyl ether for chromatography	highly flammable, irritant

*Equipment.*

Round-bottomed flask (25 ml) with rubber septum and magnetic stirrer bar × 2  
Source of dry argon (or nitrogen) as inert gas  
Syringe (5 ml) and needle  
Cannula  
Separatory funnel (100 ml)  
pH paper  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. The diphenyldiselenide should be dispensed in the fumehood. If a balance is not available within the fumehood then the diphenyldiselenide should be dispensed into a sealable container (e.g. a vial) of known mass before removal for weighing. Additionally a bleach solution should be prepared (sodium hypochlorite solution/water, 1:10. A small amount of sodium hydroxide can be added if a stronger bleaching effect is required) and placed in the fumehood. All used glassware should be placed into this once finished with in order to oxidize any trace selenides that may remain. This will

avoid any unnecessary odour. Note also that some of the early column fractions are likely need similar treatment; these can usually be easily identified by their yellow colouration. However, care should be taken to ensure no compounds that may react violently with the bleach solution are treated in this manner. It is highly recommended that the rotary evaporator used be contained within fumehood. If this is not possible a bleach solution placed in the solvent trap will help prevent any odour. In any case the rotary evaporator should be thoroughly cleaned after use.

*Procedure.* Once the glassware is dry equip a round bottomed flask (25 ml) with a magnetic stirrer bar and a rubber septum and flush this with argon, a balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 3,4,6-tri-*O*-acetyl-D-galactal (272 mg, 1 mmol), diphenyldiselenide (187 mg, 0.6 mmol), sodium azide (156 mg, 2.4 mmol) and anhydrous dichloromethane (4 ml) and stir. Next add (diacetoxyiodo)benzene (451 mg, 1.4 mmol) as a solution in anhydrous dichloromethane (1 ml) via cannula. Stir the solution at room temperature for 48 h. After this time the reaction will be complete, as evidenced by TLC analysis (diethyl ether/hexane, 1:1). The reaction is worked-up by adding dichloromethane (20 ml) and then transferring the whole solution into a separatory funnel. The solution should be washed with sodium hydrogencarbonate (2 × 8 ml, saturated solution in water), and the aqueous layers re-extracted with dichloromethane (2 × 5 ml). The combined organic layers should then be washed with distilled water until the aqueous wash is neutral (this can be checked using pH paper), dried with magnesium sulfate and removal of solvent *in vacuo* affords the crude product. The product can be purified by column chromatography (hexane/diethyl ether, 1:1). Alternatives to hexane should be considered. The compound can be isolated as a crystalline solid in 92% yield.  $[\alpha]_D^{21} = +170$  (*c* 1, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr) 2119 (N<sub>3</sub>), 1757 (CO) cm<sup>-1</sup>; mp 104–105 °C [52], 122–124 °C; [50] <sup>1</sup>H NMR [50] (300 MHz, CDCl<sub>3</sub>) 1.96, 2.05, 2.14 (3 × *s*, COCH<sub>3</sub>, 9H), 4.00 (dd, *J* = 7.0, 11.4 Hz, H-6, 1H), 4.06 (dd, *J* = 6.0, 11.4 Hz, H-6, 1H), 4.25 (dd, *J* = 5.4, 10.8 Hz, H-2, 1H), 4.66 (dt, *J* = 1.3, 6.5 Hz, H-5, 1H), 5.11 (dd, *J* = 3.2, 10.8 Hz, H-3, 1H), 5.46 (dd, *J* = 1.3, 3.2 Hz, H-4, 1H), 6.00 (d, *J* = 5.4 Hz, H-1, 1H), 7.30–7.60 (m, ArH, 5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 20.7 (3 × CH<sub>3</sub>CO), 58.8 (C-2), 61.6 (C-6), 67.3, 69.1, 71.2 (C-3, C-4, C-5), 84.2 (C-1), 127.6–134.8 (Ar), 169.8, 170.0, 170.4 (3 × CH<sub>3</sub>CO); *m/z* (CI) 471 (M + H<sup>+</sup>); Anal. Calc. for C<sub>18</sub>H<sub>21</sub>N<sub>3</sub>O<sub>7</sub>Se: C, 45.96; H, 4.50; N, 8.93. Found: C, 46.03; H, 4.53; N, 9.18%.

### 3.4.2 Glycosidation chemistry of phenyl selenoglycosides

Pinto demonstrated that phenyl selenoglycosides can be activated using silver triflate and potassium carbonate. He also showed that in the presence of a thioglycoside acting as a glycosyl acceptor these conditions activated only the phenyl selenoglycoside to give a single disaccharide product [47].

In addition the selective activation of both glycosyl trichloroacetimidates (using catalytic triethylsilyl trifluoromethanesulfonate at  $-78\text{ }^{\circ}\text{C}$ ) and glycosyl bromides (using silver trifluoromethanesulfonate and collidine) over selenoglycosides can be achieved.

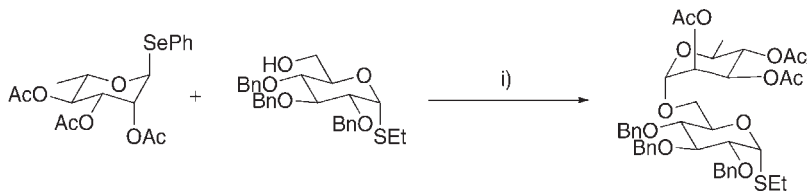
Van Boom showed that the thiophilic reagents di-*sym*-collodine perchlorate [55] and *N*-iodosuccinimide with catalytic triflic acid [56] are both excellent activators of phenyl selenoglycosides [39]. A number of other reagents developed for the activation of thioglycosides have also been shown to be effective for the activation of phenyl selenoglycosides. These include methyl triflate [28] and *N*-iodosuccinimide with catalytic trimethylsilyl triflate [79].

It has also been demonstrated that phenyl selenoglycosides can be activated by a single electron transfer reaction either chemically [80] or electrochemically [81]. However, these methods are yet to demonstrate the same synthetic utility as chemical activation.

As always in glycosidation chemistry, much thought must be given to the stereochemical outcome of the glycosidation reaction. In general it is the use of participating groups at the *O*-2 position which dominates the stereocontrol of glycosidations using a selenophenyl donor, although other strategies should be considered.

#### Method 15

Selective activation, where the glycosyl acceptor is inactive under the reaction conditions: glycosylation of ethyl 2,3,4-tri-*O*-benzyl-1-thio- $\alpha$ -glucopyranoside with phenyl 2,3,4-tri-*O*-acetyl-1-seleno- $\alpha$ -L-rhamnopyranoside [47].



i)  $\text{Ag}_2\text{CO}_3$ , AgOTf, DCM, 4Å powdered molecular sieves

*Notes and discussion.* Silver triflate and potassium or silver carbonate readily activate phenyl selenoglycosides. This procedure demonstrates the activation of phenyl selenoglycosides over thioglycosides using silver carbonate, and is from the original full paper on this topic. Note the use of silver carbonate, rather than potassium carbonate, as this improved the yield in this example.

The stereocontrol in this reaction results from the participation of an acetate on C-2 of the glycosyl donor.

*Materials.*

2,3,4-Tri- <i>O</i> -acetyl-1-seleno- $\alpha$ -L-rhamnopyranoside (107 mg, 0.25 mmol)	irritant
Ethyl 2,3,4-tri- <i>O</i> -benzyl-1-thio- $\alpha$ -glucopyranoside (124 mg, 0.25 mmol)	irritant
4 Å powdered molecular sieves (200 mg)	irritant
Anhydrous dichloromethane (8 ml, distilled from CaH <sub>2</sub> )	highly flammable, harmful
Dry silver carbonate (345 mg, 1.25 mmol)	irritant
Silver triflate (193 mg, 0.75 mmol)	irritant
Celite <sup>®</sup>	irritant
Distilled water (20 ml)	
Magnesium sulfate	irritant
Toluene and ethyl acetate for chromatography	highly flammable, harmful, irritant, may cause drowsiness/ dizziness

*Equipment.*

Bunsen burner  
 Round-bottomed flask (25 ml) with rubber septum and magnetic stirrer bar  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) as inert gas  
 Vacuum line  
 Syringe (10 ml) and needle  
 Sintered funnel  
 Separatory funnel (500 ml)  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils.

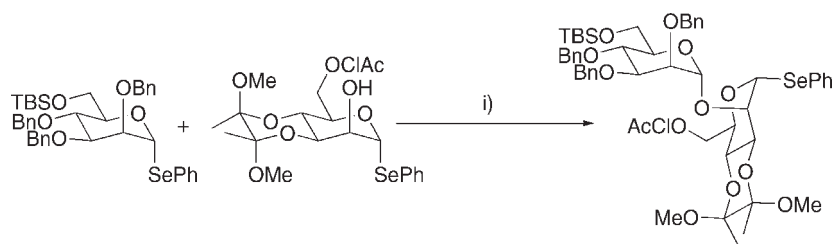
Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round bottomed flask with a magnetic stirrer bar and a rubber septum and flush it with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. To the reaction vessel add phenyl 2,3,4-tri-*O*-acetyl-1-seleno- $\alpha$ -L-rhamnopyranoside (107 mg, 0.25 mmol), ethyl 2,3,4-tri-*O*-benzyl-1-thio- $\alpha$ -glucopyranoside (124 mg, 0.25 mmol) and 4 Å powdered molecular sieves (200 mg), place on a vacuum line and dry over night. Next add anhydrous dichloromethane (8 ml) (remember to preserve the inert atmosphere) and allow the reaction to stir for 1 h. Finally, add dry silver carbonate (345 mg, 1.25 mmol) and silver triflate (193 mg, 0.75 mmol) and monitor the reaction by TLC (toluene/ethyl acetate, 5:1). Once the reaction is complete, as indicated by TLC, filter the reaction mixture through Celite<sup>®</sup> and transfer the solution to a separatory funnel. The organic layer should now be wash with distilled water (2  $\times$  10 ml), dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the crude product. The product can be purified by column chromatography (toluene/ethyl acetate, 8:1). The compound can be isolated as an oil in 80% yield.  $[\alpha]_D^{21} = +44.0$  (c 0.5, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.18 (d, *J* = 6.2 Hz, H-6, 1H), 1.31 (t, SCH<sub>2</sub>CH<sub>3</sub>, 3H), 1.98, 2.05, 2.14 (3  $\times$  s, COCH<sub>3</sub>, 9H), 2.58 (m, SCH<sub>2</sub>CH<sub>3</sub>, 2H), 3.40 (at, *J* = 18.8 Hz, H-4, 1H), 3.52 (dd, *J* = 6.4, 10.7 Hz, H-6, 1H), 3.83 (dd, *J* = 5.4, 9.2 Hz, H-2, 1H), 3.83–3.94 (m, H-3, H-6, H-5', 3H), 4.25 (ddd, *J* = 1.5, 6.4, 10.0 Hz, H-5, 1H), 4.56 (d, *J* = 11.5 Hz, PhCH<sub>2</sub>, 1H), 4.65 (d, *J* = 1.7 Hz, H-1', 1H), 4.66 (d, *J* = 11.5 Hz, PhCH<sub>2</sub>, 1H), 4.75 (d, *J* = 11.5 Hz, PhCH<sub>2</sub>, 1H), 4.77 (d, *J* = 10.8 Hz, PhCH<sub>2</sub>, 1H), 4.91 (d, *J* = 11.2 Hz, PhCH<sub>2</sub>, 1H), 4.97 (d, *J* = 10.8 Hz, PhCH<sub>2</sub>, 1H), 5.05 (at, *J* = 19.8 Hz, H-4', 1H), 5.22 (dd, *J* = 1.7, 3.5 Hz, H-2', 1H), 5.26 (dd, *J* = 3.5, 10.0 Hz, H-3', 1H), 5.39 (d, *J* = 5.4, H-1, 1H), 7.10–7.50 (m, ArH, 15H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 14.6 (SCH<sub>2</sub>CH<sub>3</sub>), 17.3 (C-6'), 20.6, 20.75, 20.8 (3  $\times$  COCH<sub>3</sub>), 23.5 (SCH<sub>2</sub>CH<sub>3</sub>), 66.3 (C-5'), 66.8 (C-6), 69.0 (C-3'), 69.7 (C-2'), 70.3 (C-5), 71.1 (C-4'), 72.3, 75.0, 75.7 (3  $\times$  PhCH<sub>2</sub>), 77.8 (C-4), 79.7 (C-2), 82.5 (C-1, C-3), 97.7 (C-1'), 127.6–138.6 (Ar), 169.7, 169.91, 169.94 (3  $\times$  CH<sub>3</sub>CO); Anal. Calc. for C<sub>41</sub>H<sub>50</sub>O<sub>12</sub>S: C, 64.21; H, 6.57. Found: C, 64.40; H, 6.80%.

### Method 16

Selective activation utilising the armed/disarmed approach, where the glycosyl donor is preferentially activated under the reaction conditions: glycosylation of (2'*S*,3'*S*) phenyl 6-*O*-chloroacetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-seleno- $\alpha$ -D-mannopyranoside with phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-seleno- $\alpha$ -D-mannopyranoside [28].





i) NIS, TMSOTf, DCM, Et<sub>2</sub>O, 4Å powdered molecular sieves, 1 h

*Notes and discussion.* This reaction demonstrates the use of *N*-iodosuccinimide (NIS) in conjunction with catalytic silyl triflate (in this case trimethylsilyl triflate). These are currently the most commonly used conditions for the activation of phenyl selenoglycosides. It is also an excellent example of reactivity tuning, and as mentioned previously, this is one of the major applications of phenyl selenoglycoside donors. In this system the butane diacetal and Chloroacetyl protecting groups disarm the acceptor electronically and conformationally. Variation of the triflate may be required in order to optimise to reaction conditions if applied to a different system.

#### *Materials.*

Phenyl 2,3,4-tri- <i>O</i> -benzyl-6- <i>O</i> - <i>tert</i> -butyldimethylsilyl-1-seleno-α-D-mannopyranoside (83 mg, 0.12 mmol)	irritant
(2' <i>S</i> ,3' <i>S</i> ) Phenyl 6- <i>O</i> -chloroacetyl-3,4- <i>O</i> -(2',3'-dimethoxybutane-2',3'-diyl)-1-seleno-α-D-mannopyranoside (53 mg, 0.104 mmol)	irritant
Dry toluene	highly flammable, harmful
4 Å powdered molecular sieves (200 mg)	irritant
Dry dichloromethane (1 ml, distilled from calcium hydride)	highly flammable, harmful
Dry diethyl ether (1 ml, distilled from sodium/benzophenone)	extremely flammable, may form explosive peroxides, harmful, may cause drowsiness/dizziness
Diethyl ether (20 ml)	extremely flammable,

	may form explosive peroxides, harmful, may cause drowsiness/dizziness
<i>N</i> -Iodosuccinimide (NIS), 122 mg, 0.54 mmol	harmful, irritant
Trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a solution in dichloromethane (50 $\mu$ l TMSOTf in 1 ml dry dichloromethane)	flammable, causes burns, highly flammable, harmful
Triethylamine (0.1 ml)	flammable, harmful, causes burns
Celite <sup>®</sup>	irritant
Sodium hydrogencarbonate (20 ml, saturated aqueous solution)	
Sodium thiosulfate (20 ml, 10% solution in water)	
Magnesium sulfate	
Petroleum ether (bp 40–60 °C) and diethyl ether for chromatography	extremely flammable, may form explosive peroxides, harmful, may cause drowsiness/dizziness

*Equipment.*

Bunsen burner  
Round-bottomed flask (5 ml) with rubber septum and magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Vacuum line  
Syringe (10 ml) and needle  
Syringe (1 ml) and needle  
Microsyringe (50  $\mu$ l) and needle  
Sintered funnel  
Separatory funnel (500 ml)  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, under an inert atmosphere. Additionally, once the glassware has been assembled and placed under an inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow it to cool, while maintaining the inert atmosphere. To the reaction vessel add phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-seleno- $\alpha$ -D-mannopyranoside (83 mg, 0.12 mmol) and (2',3',3'S) phenyl 6-*O*-chloroacetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-seleno- $\alpha$ -D-mannopyranoside (53 mg, 0.104 mmol). These should be dried by azeotropic distillation with dry toluene (this process involves adding toluene to the reaction vessel and then removing the toluene by distillation, repetition of the process removes trace water from the reagents). The reaction vessel should then be placed on a vacuum line for 4 h to complete the drying process. Next add molecular sieves (4 Å powdered, 200 mg) and dry dichloromethane/diethyl ether (1:1 ml), this will give a suspension which should be stirred for 30 min. NIS (122 mg, 0.54 mmol) and TMSOTf (10  $\mu$ l of a solution of 50  $\mu$ l TMSOTf in 1 ml dry dichloromethane) should then be added. The solution will turn dark brown immediately and should be stirred for 1 h. After this time the reaction will be complete, as evidenced by TLC analysis (petroleum ether 40/60–diethyl ether, 1:1). The reaction is worked up by the addition of triethylamine (0.1 ml) and then diethyl ether (20 ml) and the solution filtered through Celite<sup>®</sup>. The whole solution should now be transferred to a separatory funnel, and the solution washed with sodium hydrogencarbonate (20 ml, saturated aqueous solution), then sodium thiosulfate (20 ml, 10% solution in water). Drying the organic layer with magnesium sulfate, filtration and removal of the solvent *in vacuo* affords the crude product. The product can be purified by column chromatography (petroleum ether (bp 40–60 °C): diethyl ether, 3:1  $\rightarrow$  2:1). The compound can be isolated as an oil in 87% yield. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) 0.02 (s, CH<sub>3</sub>–TBS, 6H), 0.90 (s, CH<sub>3</sub>–*t*Bu, 9H), 1.32 (s, CH<sub>3</sub>–BDA, 6H), 3.22 (s, OCH<sub>3</sub>–BDA, 3H), 3.33 (s, OCH<sub>3</sub>–BDA, 3H), 3.63–3.68 (m, H-5', 1H), 3.75 (d, *J* = 10.4 Hz, H-6', 1H), 3.83 (dd, *J* = 4.3, 10.9 Hz, H-6', 1H), 3.88 (d, *J* = 14.7 Hz, ClAc, 1H), 3.92–3.99 (m, ClAc, H-2', H-3', H-4', 4H), 4.01 (dd, *J* = 1.9, 9.9 Hz, H-3, 1H), 4.07–4.13 (m, H-4, 1H), 4.30 (s, H-2, 1H), 4.31–4.36 (m, H-5, H-6, 2H), 4.44 (d, *J* = 9.5 Hz, H-6, 1H), 4.57–4.64 (m, PhCH<sub>2</sub>, 4H), 4.70 (d, *J* = 12.0 Hz, PhCH<sub>2</sub>, 1H), 4.91 (d, *J* = 10.5 Hz, PhCH<sub>2</sub>, 1H), 5.33 (s, H-1', 1H), 5.79 (s, H-1, 1H), 7.22–7.35 (m, ArH, 16H), 7.40 (d, *J* = 3.3 Hz, ArH, 2H), 7.55 (d, *J* = 2.8 Hz, ArH, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) –5.3, –5.11 (CH<sub>3</sub>–TBS), 17.7, 17.8 (CH<sub>3</sub>–BDA), 18.3 (C(CH<sub>3</sub>)<sub>3</sub>), 26.0 (CH<sub>3</sub>–*t*Bu), 40.6 (CH<sub>2</sub>–ClAc), 48.1, 48.1 (OCH<sub>3</sub>–BDA), 62.7 (C-6'), 63.5 (C-4), 63.8 (C-6), 69.7

(C-3), 70.8 (C-5), 72.0, 72.1 (PhCH<sub>2</sub>), 73.7 (C-5'), 74.7, 75.1, 79.8, (C-2', C-3', C-4'), 76.2 (C-2), 85.1 (C-1), 98.6 (C-1'), 99.8, 100.0 (C-BDA), 127.3, 127.5, 127.6, 127.7, 127.8, 128.0, 128.2, 128.3, 129.2, 129.4, 133.5, 138.6, 138.7, 138.7 (Ar{C-1, C-2, C-3, C-4, C-5, C-6}), 167.1 (CO-ClAc); HR-MS *m/z* (ESI) 1079.3251 (M + Na); C<sub>53</sub>H<sub>69</sub>O<sub>13</sub>SiSeCl requires (M + Na) 1079.3258; Anal. Calc. for C<sub>53</sub>H<sub>69</sub>O<sub>13</sub>SiSeCl: C, 60.25; H, 6.58. Found: C, 60.23; H, 6.76%.

### 3.5 GLYCALS

Over recent years, 1,5-anhydro-2-deoxy-hex-1-enitols (more commonly called glycals) have proved to be useful starting points in the stereoselective synthesis of *O*-glycosides according to a variety of differing strategies.

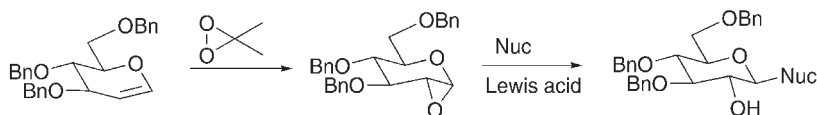
Brigl first proposed the use of 1,2-anhydrosugars (the so-called Brigl's anhydrides) as C-1 electrophiles in the first half of the last century. However, routes to these compounds were difficult and epoxidations of glycals using typical epoxidising agents were generally of such low yield that it was not until Danishefsky's application [57] of 2,2-dimethyldioxirane [58] as a very mild epoxidising agent that this methodology was truly exploited in an efficient way [59]. Thus, Lewis acid activation of e.g. the 1,2-anhydrosugar of D-glucose, stereospecifically creates 1,2-*trans* glucosides (Scheme 3.7).

The earlier advent of the Ferrier reaction [60] had already realised the potential of glycals as direct glycosyl donors. Activation of the  $\pi$ -system of 3,4,6-triacetyl-D-glucal by the presence of a Lewis acid, leads to loss of the acetate at C-3. The glycosyl cation thus formed can be intercepted by an *O*-nucleophile resulting in the stereoselective formation of a 2,3-unsaturated  $\alpha$ -glycoside (Scheme 3.8).

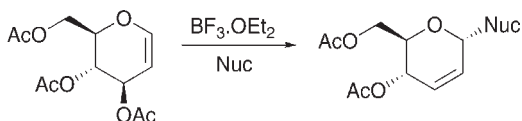
The enol ether nature of glycals has also been exploited in the synthesis of oligosaccharides containing 2-deoxy sugars. Haloglycosylation, developed by Lemieux [61] and Thiem [62], gave chemists access to 2-halo- $\alpha$ -mannosides, a process that was shown to be iterative by Friesen and Danishefsky [63] through the use of 'armed' and 'disarmed' [38] protecting groups (Scheme 3.9).

#### 3.5.1 Preparation of glycals

Zinc catalyzed reductive elimination of acetobromoglucose to give triacetyl D-glucal [64] is the most usual entry into glycal chemistry. Usefully the product can be deprotected under Zemplén conditions and the resulting trihydroxyglucal [65] can be elaborated e.g. fully protected, as its trisilyl ether by treatment with an excess of *tert*-butyldimethylsilylchloride and imidazole in DMF [66].

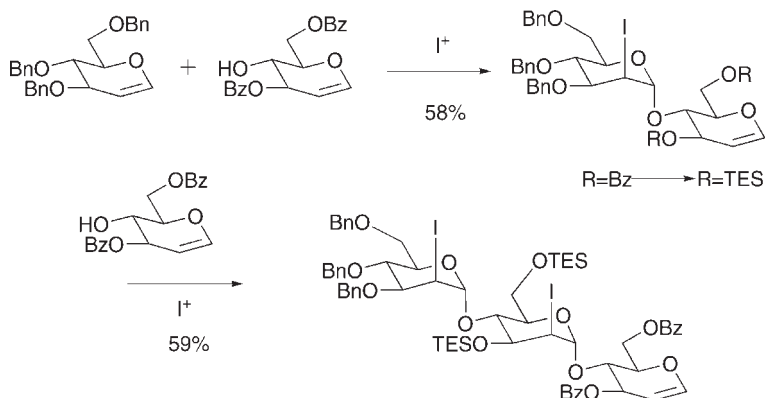


Scheme 3.7



Scheme 3.8

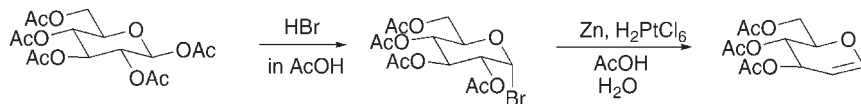
Alternative strategies have also been employed. Allose-derived glycols have been obtained through a [2,3]-sigmatropic rearrangement of an anomeric sulfoxide [57]. Less commonly, glycols have also been synthesised by reductive elimination of an anomeric sulfone [67], and by Claisen rearrangement of a vinyl glycoside, giving access to glycols having branch points at C-3 [68].



Scheme 3.9

### Method 17

Synthesis of 3,4,6-*O*-triacyl-D-glucal [64, 69].



*Notes and discussion.* The traditional (and indeed the original) method of the synthesis of glycols is via reductive elimination of an acylated glycosyl halide. This reaction leads to the formation of the acetylated glycol, which can then be de-acetylated and further functionalised. A variety of reducing conditions have been reported: zinc/acetic acid with or without platinum chloride has been most commonly

used (experimental below), but higher yields have been reported for reactions involving titanium(III) [70], and Vitamin B<sub>12</sub> catalysis [71]. This method is applicable to all cases where further functionalisation is carried out in neutral or basic conditions, e.g. benzylation, silylation. Here the preparation is described from D-glucose pentaacetate and so includes a variation on acetobromoglucose formation.

*Materials.*

1,2,3,4,6-Penta- <i>O</i> -acetyl- $\beta$ -D-glucopyranose (30 g, 76.9 mmol)	no known risk
Hydrogen bromide, 33% in acetic acid (52.5 ml)	toxic, highly corrosive
Dichloromethane for work-up (1600 ml)	risk of irreversible effects
Zinc powder (50 g, 765 mmol)	no known risk
Platinic chloride (95 mg, 0.231 mmol)	harmful dust, mutagen, corrosive, light and moisture sensitive
Acetic acid (50 ml)	flammable, causes burns
Water (50 ml)	no hazard
Petroleum ether (bp 40–60 °C) for purification	highly flammable, harmful

*Equipment.*

Bromide formation:

Two round-bottomed flasks (250 and 1000 ml) with rubber septum and magnetic stirrer bar

Magnetic stirrer

Source of dry argon (or nitrogen) as inert gas

Measuring cylinder

Reductive elimination:

Round-bottomed flask (500 ml) with rubber septum and magnetic stirrer bar

Magnetic stirrer

Source of dry argon (or nitrogen) as inert gas

Measuring cylinder

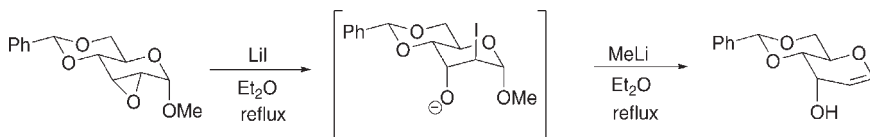
Rotary evaporator

**Procedure.** Add 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose (30 g, 76.9 mmol) portionwise over 45 min to an ice-cold solution of hydrogen bromide, 33% in acetic acid (52.5 ml). Stir the mixture for 1 h under an atmosphere of argon until TLC analysis (petroleum ether 40/60–ethyl acetate, 1:1) indicates the consumption of starting material ( $R_f$  0.4) and the formation of a major product ( $R_f$  0.5), the glycosyl bromide. Some minor hydrolysis product ( $R_f$  0.2) may also be formed. Pour the reaction mixture into vigorously stirred ice-water (500 ml) in a 1000 ml conical flask. Extract the resulting mixture with DCM (4 × 200 ml). Combine the organic layers and wash with sodium bicarbonate solution (aq., sat., 4 × 250 ml), dry with  $MgSO_4$ , filter and then concentrate *in vacuo*. The residue is 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide and can be carried forward in the synthesis without further purification.  $^1H$  NMR (400 MHz,  $CDCl_3$ ) 2.04, 2.05, 2.10, 2.10 (4 × s, 4 ×  $CH_3$ , 12H), 4.10–4.15 (m, H-6, 1H), 4.27–4.35 (m, H-5, H-6', 2H), 4.84 (dd,  $J$  = 10.2, 3.9 Hz, H-2, 1H), 5.16 (at,  $J$  = 9.8 Hz, H-4, 1H), 5.56 (at,  $J$  = 9.7 Hz, H-3, 1H), 6.61 (d, H-1, 1H).

Dissolve the crude 2,3,4,6-tetra-*O*-acetyl-1-bromo-D-glucopyranose in a mixture of acetic acid (50 ml) and water (50 ml). Add zinc powder (50 g, 765 mmol) and platinum chloride (95 mg, 0.231 mmol) to the solution, and stir the mixture under an atmosphere of argon. It is important to regularly observe the reaction, as a grey foam is formed, which could block the needle to the argon balloon, resulting in a build-up of pressure within the flask. For this reason, it is also important to use a large flask. After stirring for 1 h 30 min, TLC analysis (petroleum ether 40/60–ethyl acetate, 1:1) indicates consumption of starting material ( $R_f$  0.5) and formation of a major product ( $R_f$  0.6). Filter the reaction mixture through Celite<sup>®</sup>, and extract the filtrate with DCM (4 × 200 ml). Combine the organic layers, wash with sodium bicarbonate solution (3 × 400 ml), dry with  $MgSO_4$ , filter and concentrate *in vacuo*. Recrystallize the residue from petroleum ether 40/60 to give 3,4,6-tri-*O*-acetyl-D-glucal (24.74 g, 88% over two steps) as a white crystalline solid, mp 51–53 °C;  $[\alpha]_D^{24}$  = −17.3 ( $c$  0.95,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ ) 2.04, 2.07, 2.09 (3 × s, 3 ×  $CH_3$ , 9H), 4.19 (dd,  $J$  = 3.1 Hz, 11.8 Hz, H6, 1H), 4.23–4.27 (m, H5, 1H), 4.40 (dd,  $J$  = 5.6 Hz, H6', 1H), 4.84 (dd,  $J$  = 6.1, 3.6 Hz, H-2, 1H), 5.22 (dd,  $J$  = 5.7, 7.6 Hz, H-4, 1H), 5.32–5.35 (m, H-3, 1H), 6.46 (dd,  $J$  = 1.5 Hz, H-1, 1H).

### Method 18

Synthesis of 4,6-*O*-benzylidene-D-allal [72].



**Notes and discussion.** Further functionalisation of the glycals obtained by Method 17 above is limited to reactions that can be carried out in neutral or basic conditions, e.g. benzylation or silylation. Reactions requiring acidic catalysis are incompatible

with the acid-sensitive enol ether present in the molecule. For example, the synthesis of 4,6-*O*-benzylidene protected glycals requires a different approach. 4,6-*O*-Benzylidene-D-allal can be synthesised in a one-pot procedure from methyl 2,3-anhydro-4,6-*O*-benzylidene-D-glucopyranoside through reductive elimination of the 2-iodo species [73]. In this example, trans-diaxial opening of the 2,3-epoxide is exploited to give the 2-iodo-2-deoxy-D-altro species, which is subjected to metal-halogen exchange conditions in the same pot, giving 4,6-*O*-benzylidene-D-allal in 4 steps from commercially available methyl  $\alpha$ -D-glucopyranoside.

#### *Materials.*

Methyl 2,3-anhydro-4,6- <i>O</i> -benzylidene- $\alpha$ -D-allopyranoside (2 g, 7.57 mmol)	assume toxic
Anhydrous lithium iodide (3.04 g, 22.7 mmol)	harmful
Anhydrous diethyl ether (50 ml)	extremely flammable, may form explosive peroxides
Methyl lithium (1.6 M in diethyl ether, 18.9 ml, 30.3 mmol)	extremely flammable, corrosive, not yet fully tested
Isopropanol (8 ml)	highly flammable
Diethyl ether for work-up (150 ml)	extremely flammable, may form explosive peroxides
Water (400 ml)	no hazard
Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, harmful

#### *Equipment.*

Two neck round-bottomed flask (100 ml) with two rubber septa and magnetic stirrer bar  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) as inert gas  
 Gas-tight syringe (20 ml)  
 Rotary evaporator  
 Flash chromatography equipment



*Procedure.* Stir the methyl 2,3-anhydro-4,6-*O*-benzylidene- $\alpha$ -D-allopyranoside (2 g, 7.57 mmol) and anhydrous lithium iodide (3.04 g, 22.7 mmol) in anhydrous diethyl ether (50 ml), and then reflux the resulting mixture under an atmosphere of argon. After 30 min, TLC analysis (petroleum ether 40/60–ethyl acetate, 1:1) should indicate the complete consumption of starting material ( $R_f$  0.5) and the formation of a single product ( $R_f$  0.6), methyl 4,6-*O*-benzylidene-2-deoxy-2-iodo- $\alpha$ -D-altropyranoside,  $[\alpha]_D^{21} = +30.8$  ( $c$  0.925,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 3.07 (d,  $J = 7.0$  Hz, OH, 1H), 3.45 (s,  $\text{OCH}_3$ , 3H), 3.91 (at,  $J = 10.1$  Hz, H-6, 1H), 4.10–4.24 (m, H-5, 1H), 4.25–4.31 (m, H-3, 1H), 4.29–4.37 (m, H-2, 1H), 4.34–4.39 (m, H-2, 1H), 4.43 (dd,  $J = 2.6, 9.9$  Hz, H-4, 1H), 5.03 (d,  $J = 0.9$  Hz, H-1, 1H), 5.69 (s, PhCH, 1H), 7.34–7.54 (m,  $5 \times \text{Ar-H}$ , 5H).

Cool the reaction mixture to room temperature, and add methyl lithium (1.6 M in diethyl ether, 18.9 ml, 30.3 mmol) using the gas-tight syringe under an inert atmosphere. Reflux the mixture again under an atmosphere of argon. After 2 h, TLC analysis (petroleum ether 40/60–ethyl acetate, 1:1) should show consumption of the intermediate ( $R_f$  0.6) and formation of a major product ( $R_f$  0.7), 4,6-*O*-benzylidene-D-allal, and a minor product ( $R_f$  0.3). Quench the reaction mixture carefully by dropwise addition of isopropanol (8 ml) followed by dropwise addition of water (20 ml). Add diethyl ether (150 ml) and water (80 ml) and then transfer to a separatory funnel. Separate the organic layer, wash with water ( $3 \times 100$  ml), dry with  $\text{MgSO}_4$ , filter, and concentrate *in vacuo*. Purify the residue by flash chromatography (petrol/ethyl acetate, 2:1  $\rightarrow$  1:1) to afford 4,6-*O*-benzylidene-D-allal (1.42 g, 80%) as a white crystalline solid, mp 79–81 °C (ethyl acetate/petrol);  $[\alpha]_D^{25} = +196.7$  ( $c$  0.94,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 2.50 (s, OH-3, 1H), 3.83–3.89 (m, H-4, H-5, 2H), 4.22 (dd,  $J = 5.2, 10.3$  Hz, H-6, 1H), 4.25–4.30 (m, H-3, 1H), 4.49 (dd,  $J = 5.3$  Hz, H-6', 1H), 5.04 (at,  $J = 5.9$  Hz, H-2, 1H), 5.68 (s, PhCH, 1H), 6.47 (d,  $J = 6.0$  Hz, H-1, 1H), 7.37–7.54 (m,  $5 \times \text{Ar-H}$ , 5H).

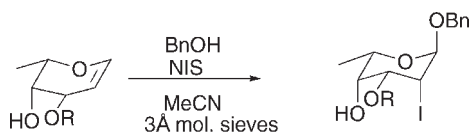
### 3.5.2 Use of glycols as direct glycosyl donors: haloglycosylation

Various pathways for the electrophilic addition to a glycol followed by nucleophilic opening of the resulting halonium ion can account for the different product distributions obtained from these reactions [74]. Irreversible formation of the halonium ion and subsequent nucleophilic displacement leads to the  $\beta$ -*gluco* compound, while reversible formation of the halonium ion followed by slow nucleophilic *trans* diaxial opening leads to the  $\alpha$ -*manno* compound. Common ' $\text{I}^+$ ' sources used to synthesise  $\alpha$ -*manno* species include *N*-iodosuccinimide (NIS), and iodonium di-*sym*-collidine perchlorate (IDCP). An iodonium source is particularly favoured over a bromonium source, as this more readily allows further subsequent functionalisation of the product often via radical chemistry. The yields of

haloglycosylations are generally between 50% and 70%, with product distributions  $\leq 7:1$  in favour of the  $\alpha$ -*manno* diastereomer.

### Method 19

Synthesis of benzyl 3-*O*-(4-methoxybenzyl)-2,6-dideoxy-2-iodo- $\alpha$ -L-talopyranoside [75].



R = 4-methoxybenzyl

*Notes and discussion.* The example given here is a typical procedure for iodoglycosylation. In this specific case, only one diastereomer is produced, resulting from *trans*-diaxial opening of the  $\beta$ -iodonium ion.

### Materials.

1,5-Anhydro-2,6-dideoxy-3- <i>O</i> -(4-methoxybenzyl)-L-lyxo-hex-1-enitol (400 mg, 1.6 mmol)	assume toxic
Anhydrous acetonitrile (1 ml)	highly flammable, toxic
Anhydrous benzyl alcohol (1 ml, 9.6 mmol)	irritant, harmful
<i>N</i> -iodosuccinimide (540 mg, 2.4 mmol)	irritant, harmful
Powdered 3 Å molecular sieves (200 mg)	irritant dust
Dichloromethane for work-up (50 ml)	risk of irreversible effects
Sodium thiosulfate solution (50 ml of a 10% aqueous solution)	irritant
Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, harmful

### Equipment.

Round-bottomed flask (10 ml) with rubber septum and magnetic stirrer bar  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) as inert gas  
 Syringe (1 ml) and needles  
 Rotary evaporator  
 Flash chromatography equipment

**Procedure.** Dissolve 1,5-anhydro-2,6-dideoxy-3-*O*-(4-methoxybenzyl)-L-lyxo-hex-1-enitol (400 mg, 1.6 mmol) in anhydrous acetonitrile (1 ml) in a round bottomed flask covered with aluminium foil to exclude light from the reaction. Add to the solution the anhydrous benzyl alcohol (1 ml, 9.6 mmol), *N*-iodosuccinimide (540 mg, 2.4 mmol) and 3 Å molecular sieves (200 mg). Stir the resulting mixture under an atmosphere of argon in the dark for 12 h. Dilute the mixture with dichloromethane (50 ml), and filter. Wash the filtrate with sodium thiosulfate solution (50 ml of a 10% aqueous solution), dry with MgSO<sub>4</sub>, filter and concentrate the filtrate *in vacuo*. Purify the residue by flash chromatography (petroleum ether 40/60–ethyl acetate, 3:1) to give benzyl 3-*O*-(4-methoxybenzyl)-2,6-dideoxy-2-iodo- $\alpha$ -L-talopyranoside (520 mg, 67%) as a yellow syrup; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 1.30 (d, *J* = 6.6 Hz, H-6, 3H), 1.70 (br s, OH-4, 1H), 3.75 (s, OCH<sub>3</sub>, 3H), 3.90 (br d, *J* = 1.3, 3.8 Hz, H-4, 1H), 4.00 (dq, *J* = 1.3, 6.6 Hz, H-5, 1H), 4.30 (br d, *J* = 4.4, <1.0 Hz, H-2, 1H), 4.40–4.70 (4 × d, *J* = 11.6, 12.0 Hz, PhCH<sub>2</sub>, 4H), 5.40 (br s, H-1, 1H), 6.80–7.40 (m, Ar-H, 9H).

### 3.5.3 Using glycals to form epoxide glycosyl donors: 1,2-anhydrosugar glycosylation

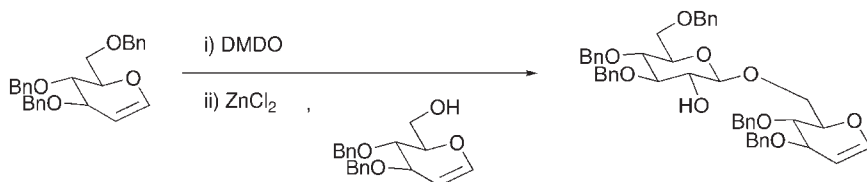
Not until Halcomb and Danishefsky [76] developed a high yielding method for the conversion of glycals to the corresponding 1,2-anhydrosugars did 1,2-anhydroglycosylation become a viable route to 1,2-*trans* oligosaccharides. The key was the use of the epoxidising agent 2,2-dimethyldioxirane (DMDO) [58], which was found to react smoothly with a variety of glycals to give almost exclusively  $\alpha$ -1,2-anhydrosugars in high yield. Glycosylation using zinc chloride catalysis to activate these epoxides resulted in highly stereospecific S<sub>N</sub>2-type opening of the epoxide.

A vital strategic feature of this methodology is that ready iteration is possible; glycosylation of a partially protected glycal can be followed by further DMDO treatment. This allows effective non-reducing end to reducing end extension. Moreover, the exposure of the OH-2 hydroxyl following glycosylation allows differentiation at OH-2 as an inherent advantage and makes this a very useful method for the synthesis of oligosaccharides that contain a branch point at C-2.

Some drawbacks should also be noted: the limited scale on which the 1,2-anhydrosugars can be synthesised. This is a result of the very dilute solutions of DMDO in acetone that may be prepared [58] (typically 0.05–0.1 M) and as a result very large volumes are required for even fairly modest scale glycosylations. Also, typically only the  $\alpha$ -1,2-anhydrosugar can be synthesised, normally giving rise to only  $\beta$ -*gluco* products (although manipulations are possible: e.g. oxidation and reduction of the OH-2 hydroxyl exposed following glycosylation provides ready access to  $\beta$ -*manno* products—see Chapter 8).

*Method 20*

Synthesis of 6-*O*-(3,4,6-tri-*O*-benzyl- $\beta$ -D-glucopyranosyl)-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-D-arabino-hex-1-enitol [76].



*Notes and discussion.* Note that this reaction has iterative potential—the product of the reaction can be subjected to further epoxidation conditions. The DMDO solution in acetone can be readily prepared using Oxone to oxidise acetone followed by distillation according to Murray's original method [58] and stored at  $-20\text{ }^{\circ}\text{C}$  for periods of weeks. Note that large volumes of the DMDO solution are often required: here 24 ml is used to produce just 45 mg of epoxide in the first stage.

*Materials.*

3,4,6-Tri- <i>O</i> -benzyl-D-glucal (43 mg, 0.10 mmol)	unknown risk
3,4-Di- <i>O</i> -benzyl-D-glucal (55.8 mg, 0.17 mmol)	unknown risk
Dichloromethane, anhydrous (1 ml)	risk of irreversible effects
Dimethyldioxirane [58] (0.05 M in acetone, 24 ml, 0.12 mmol)	assume toxic and corrosive
Tetrahydrofuran	highly flammable, harmful
Zinc chloride (1.0 M in diethyl ether)	flammable, harmful, irritant
Ethyl acetate and petroleum ether (bp $40\text{--}60\text{ }^{\circ}\text{C}$ ) for work-up and chromatography	highly flammable, irritant, harmful

*Equipment.*

Round-bottomed flask (100 ml) with rubber septum and magnetic stirrer bar  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) as inert gas  
 Syringe (25 ml) and needle  
 Rotary evaporator  
 Flash chromatography equipment

*Procedure.* Dissolve 3,4,6-tri-*O*-benzyl-D-glucal (43 mg, 0.1 mmol) in anhydrous dichloromethane (1 ml), and cool the resulting solution to 0 °C. Add a solution of dimethyldioxirane in acetone (0.05 M in acetone, 24 ml, 0.12 mmol) dropwise, and stir at 0 °C for 1 h. Evaporate the acetone with a stream of dry inert gas and dry the residue *in vacuo* to afford 1,2-anhydro-3,4,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranose (45 mg, quant.). This epoxide can be used without further purification;  $[\alpha]_D^{25} = +29.2$  (*c* 0.96, CHCl<sub>3</sub>); <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) 3.10 (d, *J* = 2.0 Hz, 1H), 3.60–3.85 (m, 4H), 3.99 (d, *J* = 7.6 Hz, 1H), 4.50–4.88 (m, 6H), 5.00 (br d, *J* = 2.0 Hz, 1H), 7.10–7.40 (m, 15H).

Dissolve the 1,2-anhydro-3,4,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranose (49.4 mg, 0.114 mmol) in 0.15 ml of tetrahydrofuran, and cool the resulting solution to –78 °C. Add a solution of the 3,4-di-*O*-benzyl-D-glucal (55.8 mg, 0.17 mmol) in tetrahydrofuran (0.15 ml). Next add, dropwise, a solution of zinc chloride (1.0 M) in diethyl ether (0.25 ml, 0.25 mmol). Stir the reaction mixture at –78 °C under an atmosphere of argon for 1 h, and then allow it to warm to room temperature and stir at room temperature for 18 h. After this time, pour the reaction mixture into sodium bicarbonate solution (25 ml saturated aqueous) and extract with ethyl acetate (3 × 10 ml). Combine the organic layers, dry with MgSO<sub>4</sub>, filter and concentrate *in vacuo*. Purify the residue purified by flash chromatography (petroleum ether 40/60–ethyl acetate, 7:3) to give 6-*O*-(3,4,6-tri-*O*-benzyl- $\beta$ -D-glucopyranosyl)-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-D-arabino-hex-1-enitol (48.7 mg, 56%);  $[\alpha]_D^{25} = -5.9$  (*c* 2.97, CHCl<sub>3</sub>); <sup>1</sup>H NMR (490 MHz, CDCl<sub>3</sub>) 2.51 (s, 1H), 3.46 (m, 1H), 3.57–3.64 (m, 3H), 3.68–3.76 (m, 2H), 3.77 (dd, *J* = 5.8, 7.5 Hz, 1H), 3.87 (dd, *J* = 7.1, 11.9 Hz, 1H), 4.16–4.21 (m, 3H), 4.29 (d, *J* = 7.3 Hz, 1H), 4.52–4.72 (m, 6H), 4.80–4.87 (m, 3H), 4.91 (dd, *J* = 2.9, 6.2 Hz, 1H), 7.17–7.41 (m, 25H).

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# Synthesis and Activation of Carbohydrate Donors: Thioglycosides and Sulfoxides

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## 4.1 INTRODUCTION

The successful application of a protecting group for temporarily masking the anomeric centre in a carbohydrate building block during oligosaccharide synthesis depends on its stability to various reaction conditions as well as its amenability to straight forward chemoselective transformations when necessary [1]. An alkyl- or arylthio group placed at the anomeric centre satisfies most of these requirements; it is therefore of no surprise that thioglycosides are amongst the most popular classes of glycosyl donor compounds employed in oligosaccharide synthesis. Several reviews on the use of thioglycosides have appeared over the past decade (see Refs. 2–9), most of them dealing mainly with methods for activating thioglycosides in oligosaccharide assembly. While these reviews summarise methods available for the preparation as well as activation of thioglycosides, practical aspects have not been covered in any detail. The present review is designed to fill this gap in particular, and provides a perspective of reliable practical methods available from the literature to date. In addition, as thioglycosides form valuable precursors for glycosyl sulfoxides, preparation and activation of the latter are also described.

## 4.2 THIOGLYCOSIDES

### 4.2.1 Preparation

A number of methods are available for preparing thioglycosides, those utilising per-*O*-acetylated carbohydrates or acetylated glycosyl halides as the starting material being the most popular (for a summary of methods available see Table 4.1) [3, 6, 10].



The method of choice will depend upon the type of thioglycoside, namely, alkyl- or arylthioglycoside, which is to be prepared. Methods satisfactory for the preparation of both of these types of thioglycosides are outlined below.

The method based on the  $S_N2$  displacement of a per-*O*-acylated glycosyl halide by thiophenoxide, first used by Fischer and Delbruck [11], later modified by Schneider *et al.* [12] and Purves [13, 14] (see also Refs. 15–20) and further improved by Bogusiak and Szeja [21] and Tropper *et al.* [22, 23] continues to be of good practical value for preparing arylthioglycosides from acetylated glycosyl halides of mono- and disaccharides. Formation of by-products from partial (or complete) de-*O*-acylation due to the basicity of the alkoxide reagent is not significant when thiophenol (or substituted thiophenols) is used as nucleophile under phase-transfer conditions [21–23], as detailed in the procedure described below (see Method 1). Due to the strong basicity of the thiolates derived from alkyl mercaptans, this method is not well suited for converting acetohalosugars to the corresponding alkylthioglycosides (the reaction often results in the loss of acetyl groups necessitating an additional acetylation step in the synthetic sequence [24]). However, methods described later in this article provide practical alternatives.

Methods using per *O*-acetylated carbohydrates and a mercaptan in the presence of an acid catalyst, [25–35] although commonly used in the past, have often led to the formation of considerable amounts of 1,2-*cis*-linked thioglycosides as by-products [36]. Such methods are therefore not described herein, although they may be of considerable value for making thioglycosides of carbohydrates bearing acid sensitive functions such as a uronolactone ring or uronate ester group [37]. Use of  $BF_3 \cdot OEt_2$  in

**Table 4.1**

Methods for the preparation of per-*O*-acylated thioglycosides

Catalyst	References
<i>Using acetohalosugars, thiol and alkali/alkaline salts</i>	
Preformed thiolate or thiol + alkali	11–20
Phase transfer catalyst	21–23
<i>Using per-O-acetylated carbohydrates, thiol and a Lewis acid</i>	
$BF_3 \cdot OEt_2$	25 and references cited therein
$ZnCl_2$	26–31
$SnCl_4$	28, 32, 33
$ZrCl_4$	34
$FeCl_3$	35
<i>Using per-O-acetylated carbohydrates, silylated thiol and a Lewis acid</i>	
$ZnI_2$ , $Bu_4NI$	38
$BF_3 \cdot OEt_2$	36
$I_2$	10
<i>Using per-O-acetylated aldoses, disulfide and a Lewis acid</i>	
$I_2$ , $(Me_3Si)_2$	10

the above transformation usually requires reaction times ranging from 2 to 46 h [37] but the iodine-catalysed reactions are extremely fast (usually of the order of 5 min or less [10]). It is therefore important to monitor the reaction closely, as extended reaction times can lead to anomerisation of the initially formed 1,2-*trans*-linked product. As a modification to this procedure, and to reduce the side reactions mentioned above, use of the *S*-trimethylsilyl derivative of the thiol instead of the free thiol itself was subsequently introduced [36, 38]. As the trimethylsilylthioethers are generally expensive, a more recent method that makes use of a significantly cheaper reagent system, consisting of hexamethyldisilane, dialkyl/diarylsulfide and iodine, described by Kartha and Field [10] is detailed in this chapter.

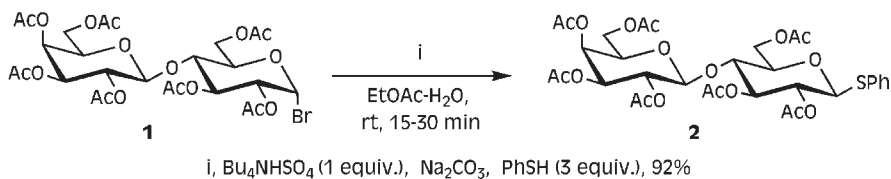
Another method for synthesising thioglycosides described here in detail is a two-step procedure consisting of the conversion of the readily available 1,2-*cis*-linked per-*O*-acetylated glycosyl halides to their corresponding 1,2-*trans*-linked glycosyl thioacetate followed by alkylation/arylation of the thiol generated *in situ* by selective de-*S*-acetylation [39]. This method is extremely versatile because a variety of alkyl/aryl (or substituted alkyl/aryl) groups can be introduced on to the sulfur in the second step, which is limited perhaps only by the choice of the reagent available for the latter alkylation/arylation step. Usually, an alkyl/aryl (or substituted alkyl/aryl) halide having the desired alkyl/aryl residue is used as the alkyl/aryl donor molecule. The method is also well-suited for the preparation of *S*-linked disaccharides in which case the alkyl/aryl halide in the above may be replaced with the desired sugar moiety bearing a halide or triflate leaving group at the desired position [40].

Other literature methods not described in detail here, utilise glycosyl sulfenate [41], thiopseudourea [42], xanthate [42, 43], thiocyanate [44, 45] and dithioacetal [44, 46] intermediates or rely on radical addition to thiohemiacetals [47, 48] or thermal decomposition of diazo compounds prepared by reacting 1-thioaldoses and aryl diazonium salts [44]. Some of these procedures have specific advantage in some specific cases, e.g. the dithioacetal to thioglycoside route is valuable for preparing 1-thio-hexofuranosides [46, 49].

## 4.2.2 Procedures

### Method 1

Preparation of phenyl hepta-*O*-acetyl-1-thio- $\beta$ -D-lactoside (**2**) from acetobromolactose (**1**) [22].



*Notes and discussion.* The method is applicable to the preparation of aryl- or substituted arylthioglycosides of most mono- and disaccharides and gives the corresponding thioglycosides with 1,2-*trans*-configuration. Glycosyl halides without participating groups next to the anomeric centre would, on the other hand, lead to products with inverted configuration at the anomeric centre, e.g. in the case of preparation of sialosides. The fact that ethyl acetate is a non-participating solvent (and is less dense than water, therefore convenient for withdrawing aliquots for monitoring the reaction by TLC) makes it the solvent of choice for the reaction. Other solvents such as dichloromethane and toluene can also be used with similar efficiency, but use of the former has in some instances led to reduced yields [22] due to the formation of side products (arising from dichloromethane acting as a substrate in the nucleophilic substitution). Likewise, although other phase transfer reagents such as  $\text{Bu}_4\text{NBr}$  and  $\text{Bu}_4\text{NI}$  can also be used instead of  $\text{Bu}_4\text{NHSO}_4$ , best results are reportedly obtained on use of the latter. Reactions are generally complete in 30 min but confirmation by NMR may be necessary if the glycosyl halide and the thioglycoside happen to have identical  $R_f$  values on TLC.

#### *Materials.*

Acetobromolactose ( <b>1</b> )	irritant
Ethyl acetate, 1 ml/100 mg of <b>1</b>	flammable, irritant
$\text{Na}_2\text{CO}_3$ , aq. 1 M, 1 ml/100 mg of <b>1</b>	irritant
$\text{Bu}_4\text{NHSO}_4$ , 1 mol equiv.	irritant
Thiophenol, 3 equiv.	stench, irritant
$\text{NaOH}$ , aq., 1 M	irritant
Distilled water	
$\text{Na}_2\text{SO}_4$ , granular, anhydrous	irritant

EtOAc/*n*-hexane (1:3–1:1) or an appropriate combination of EtOAc/toluene, for chromatography and column chromatographic grade silica gel.

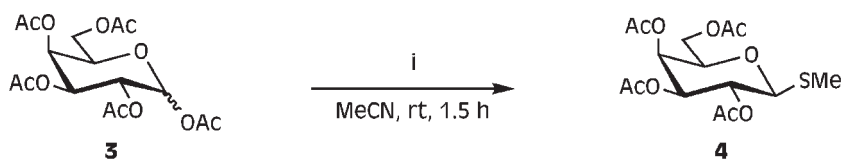
*Equipment.* The experiment should be conducted in a well-ventilated fume hood. The apparatus used and any waste solvents may be treated with commercially available bleach (aq.  $\text{NaOCl}$ ) prior to washing/disposal.

- Round bottomed or Erlenmeyer flask with magnetic stirring bar
- Magnetic stirrer
- Pipette or syringe
- Separatory funnel
- Rotary evaporator
- Column chromatography equipment

*Procedure.* Add aqueous sodium carbonate solution and thiophenol to a stirred solution of acetobromolactose and  $\text{Bu}_4\text{NHSO}_4$  in EtOAc at rt and continue stirring until the reaction is complete. Dilute the reaction mixture with EtOAc and wash the resulting solution successively with aq. NaOH and water in a separatory funnel. Back-extract the aqueous washings with additional quantities of EtOAc each time to avoid loss of any product during extraction. Dry the combined organic extract (anhydrous  $\text{Na}_2\text{SO}_4$ ), filter, concentrate to dryness under reduced pressure and purify by chromatography to afford the crystalline title compound in approximately 90% yield. Mp  $165\text{--}167\text{ }^\circ\text{C}$  (EtOH);  $[\alpha]_{\text{D}} = -18.2$  ( $c$  1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\delta\text{CDCl}_3$ ) 1.94–2.12 (7s, 21H,  $7 \times \text{COCH}_3$ ); 4.44 (d, 1H,  $J_{1',2'} = 7.9$  Hz,  $H\text{-}1'$ ); 4.65, (d, 1H,  $J_{1,2} = 10.1$  Hz,  $H\text{-}1$ ); 7.27–7.31 (m, 3H, ArH) and 7.44–7.47 (m, 2H, ArH).

### Method 2

Preparation of methyl tetra-*O*-acetyl-1-thio- $\beta$ -D-galactopyranoside (**4**) from penta-*O*-acetyl galactopyranose (**3**) [10].



i,  $\text{Me}_3\text{SiSiMe}_3$  (0.55 mol equiv.),  $\text{Me}_2\text{S}_2$  (0.55 mol equiv.),  $\text{I}_2$  (1.5 mol equiv.), >90%

*Notes and discussion.* The  $\alpha$ - and  $\beta$ -anomers of the per-*O*-acetyl glycoses are equally effective in this process. The method can be extended to the preparation of ethyl/phenyl/other thioglycosides, by using the corresponding disulfide in place of  $\text{Me}_2\text{S}_2$  as used in the present method. Although dry solvent is desirable, it has been found that the reaction can be carried out very satisfactorily in HPLC grade solvent. The reaction should be monitored by TLC and the reaction mixture should not be left for longer periods.

### Materials.

Penta- <i>O</i> -acetyl-D-galactopyranose ( <b>3</b> )	irritant
$\text{CH}_2\text{Cl}_2$ , 1 ml/100 mg of <b>3</b>	irritant
Hexamethyldisilane, 0.55 mol equiv.	irritant
Methyl disulfide, 0.55 mol equiv.	stench, irritant
Iodine, 1.5 mol equiv.	corrosive, irritant
$\text{Na}_2\text{S}_2\text{O}_3$ , aq., 10%	irritant

*Notes and discussion.* As the reaction conditions are very mild virtually all protecting groups should be compatible with this reaction and may be limited only by compatibility with the preparation of the precursor halide. Glycosyl chlorides

may also be used instead of bromides but longer reaction times or reflux temperatures (in which case 1,2-dichloroethane could be used as solvent for the reaction) may be necessary. Faster rates for the first reaction can also be achieved by carrying it out in acetone at rt under homogeneous conditions [51]. In such cases the reaction mixture may turn very dark in colour but this usually does not affect the quality of the product obtained after work-up and column chromatography [52]. Another alternative is to perform the reaction (step 1) under phase transfer conditions as explained in Method A [22]. Glycosyl thioacetates typically possess good shelf lives at rt but may be best stored in a fridge or freezer until they are required for further transformations. The procedure given here (for step 1) has been adapted from a procedure described for the conversion of acetochlorosialic acid methyl ester to the corresponding glycosyl *S*-acetate by Hasegawa *et al.* [50].

The second step described here is a modified literature procedure reported by Bennet *et al.* [39] using benzylamine instead of diethylamine. In this method formation of benzamide by the transfer of the *S*-acetyl group to benzylamine can be followed by TLC.

#### *Materials.*

Acetobromogalactose ( <b>5</b> )	irritant
Dry CH <sub>2</sub> Cl <sub>2</sub> , 1 ml/100 mg of <b>5</b>	irritant
KSac (4–5 mol equiv.)	stench, irritant
Benzylamine (2.2 mol equiv.)	irritant
Benzyl bromide (1.1 mol equiv.)	lachrymator
Dry, oxygen-free THF (1 ml/100 mg of <b>6</b> )	irritant
Triethylamine	irritant
Na <sub>2</sub> CO <sub>3</sub> , aq., 10%	irritant
Dilute aq. HCl	corrosive
Distilled water	
Sodium sulfate, granular, anhydrous	irritant

EtOAc/*n*-hexane (1:3–1:1) for chromatography and column chromatographic grade silica gel.

#### *Equipment.*

Round bottomed flask with magnetic stirring bar  
Magnetic stirrer  
Pipette or syringe

Separatory funnel  
Rotary evaporator  
Column chromatography equipment

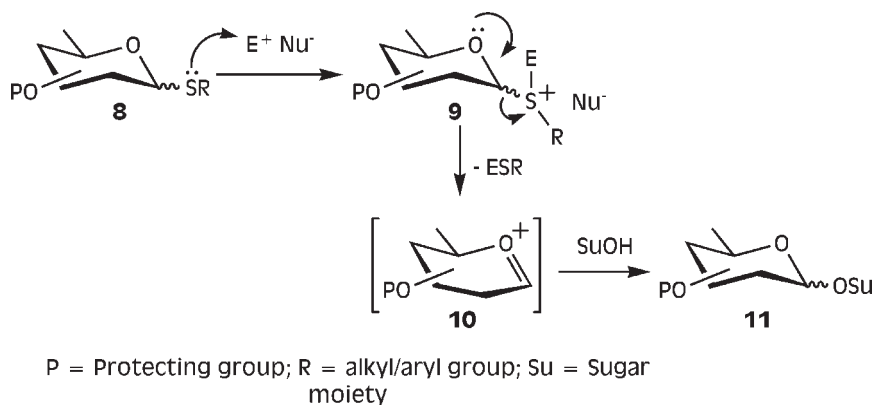
### *Procedure.*

*Step 1:* Add potassium thioacetate (5.37 g, 47 mmol) to a solution of **5** (4.11 g, 10 mmol) in dry DCM (40 ml) and stir the mixture at rt until (usually overnight) all of the starting material **5** is converted to **6** as shown by TLC (EtOAc/*n*-hexane, 2:3). Dilute the reaction mixture with DCM and wash (twice) the solution with water in a separatory funnel to remove the salts. [If strong stench persists, wash the DCM solution with aq. Na<sub>2</sub>CO<sub>3</sub> solution (twice) and water successively.] Dry the organic extract (anhydrous Na<sub>2</sub>SO<sub>4</sub>; any residual smell can be removed by treatment with activated charcoal at this stage), concentrate to a small volume under diminished pressure and chromatograph on a column of silica gel using EtOAc/*n*-hexane (1:3–2:3) as the eluent to yield pure **6** as a white solid. Mp 111–113 °C (from EtOH); [ $\alpha$ ]<sub>D</sub> = +31.6 (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (δCDCl<sub>3</sub>) 1.99–2.16 (4s, 12H, 4 × COCH<sub>3</sub>); 2.40 (s, 3H, SCOCH<sub>3</sub>); 5.12 (dd, 1H, *J*<sub>2,3</sub> = 9.5 Hz, *J*<sub>3,4</sub> = 3.5 Hz, *H*-3); 5.23–5.36 (m, 2H, *H*-2 and *H*-1); <sup>13</sup>C NMR (δCDCl<sub>3</sub>) 20.6; 20.7 (× 2); 20.8 (4 × OCOCH<sub>3</sub>); 30.1 (SCOCH<sub>3</sub>); 80.7 (*C*-1); 169.9; 170.2; 170.5; 170.7 (4 × OCOMe); 192.5 (SCOMe).

*Step 2:* Add benzyl bromide to a stirred solution of **6**, (obtained as described above), and benzylamine in THF at rt. Continue stirring until TLC (EtOAc/*n*-hexane, 2:3) shows completion of the reaction. Add Et<sub>3</sub>N and, after stirring for a few minutes, dilute the reaction mixture with DCM and wash successively with cold dilute aq. HCl (twice), aq. Na<sub>2</sub>CO<sub>3</sub> and water. Dry the organic extract (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrate it to a small volume and chromatograph on a column of silica gel using EtOAc/*n*-hexane, 1:3–2:3 as eluent. The product (**7**) is obtained as a white solid. Mp 95–96 °C; [ $\alpha$ ]<sub>D</sub> = –66.5 (*c* 0.9, CHCl<sub>3</sub>); <sup>1</sup>H NMR (δCDCl<sub>3</sub>) 4.29 (d, 1H, *J*<sub>1,2</sub> = 10 Hz, *H*-1).

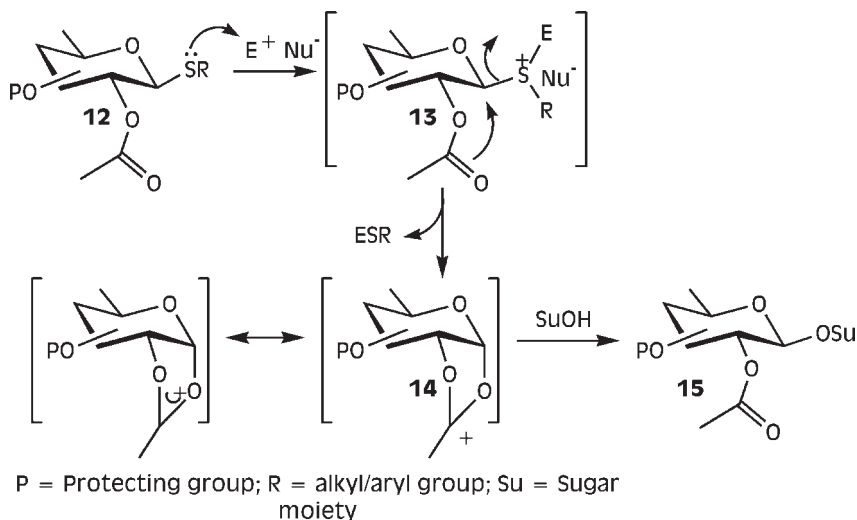
## 4.2.3 Activation

Thioglycoside glycosyl donors can be activated either directly (one step) or indirectly, (two steps) (see Figures 4.1–4.3), prior to glycosyl bond formation. There are a number of efficient ways for effecting either type of activation. In the direct method, thioglycosides are treated with an activating agent in the presence of a glycosyl acceptor to facilitate a one-step glycosylation reaction. Thus, addition of an *S*-specific electrophilic agent to a solution of the thioglycoside (**8** having a non-participating group at *C*-2/**12** having a participating group at *C*-2) in a suitable solvent results in the formation of the highly unstable sulfonium salt **9/13** (Figures 4.1 and 4.2). Displacement of the aglycon-derived sulfonyl compound, assisted by the lone pair of electrons on the ring oxygen in the case of **9** (Figure 4.1) or by the anchimeric



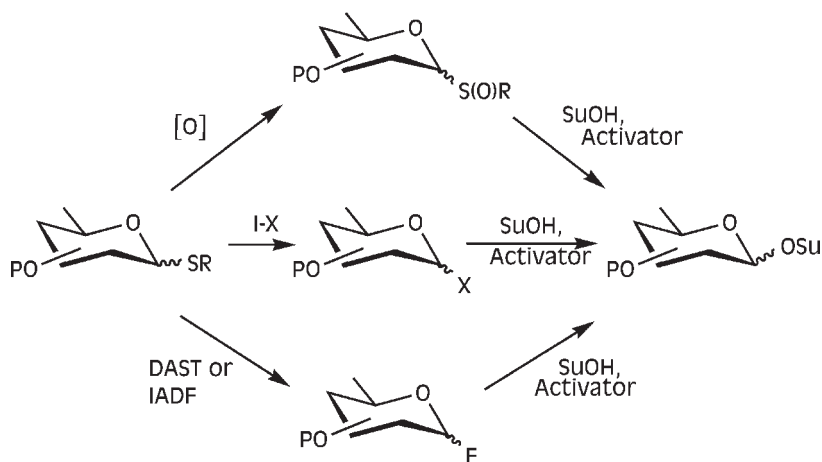
**Figure 4.1** Direct activation of thioglycosides without neighbouring group participation.

assistance from the participating group at C-2 in the case of **13** (Figure 4.2), quickly follows giving the oxocarbenium ion **10** (in the case of **9**) or the resonance stabilised cyclic oxocarbenium ion **14** (in the case of **13**). Subsequent reaction of the acceptor sugar alcohol with **10/14** affords the product **11/15**. Thus formation of **15** is stereospecific. In the indirect method thioglycosides are converted to intermediate compounds bearing a different leaving group (i.e. glycosyl halides or sulfoxides) which can then be activated by an alternative method (Figure 4.3). This latter possibility adds an extra dimension to the versatility of thioglycosides and is extremely useful in many instances. Thus on treatment with I–Br/I–Cl [53]



**Figure 4.2** Direct activation of thioglycosides with neighbouring group participation.





P = Protecting group; R = alkyl/aryl group; X = Br/Cl; Su = Sugar moiety

**Figure 4.3** Indirect activation of thioglycosides for O-glycosylation.

thioglycosides yield the corresponding glycosyl bromides/glycosyl chlorides, and with DAST/hypervalent iodoarene difluoride [54, 55] yield the corresponding glycosyl fluorides. The resulting compounds can be activated by various agents such as iodine, iodine halides and heavy metal salts for coupling with the desired acceptor molecule [53, 56, 57]. Thioglycosides can also be coupled with glycosyl acceptors *via in situ* generation of glycosyl halides using, e.g. bromine–AgOTf system [58] or simply I–Br [53] in one pot. Furthermore, thioglycosides can be conveniently oxidised to highly reactive glycosyl sulfoxides that can subsequently be activated by reagents such as triflic anhydride for coupling with acceptor substrates to yield oligosaccharides (see next section for more details on the preparation and activation of glycosyl sulfoxides). Both direct and indirect methods for activation rely on the intrinsic soft nucleophilic characteristic of the sulfur atom in the thioglycosides and in particular its halophilicity. It is therefore of no surprise that many of the more effective activating species are indeed halogen-based (see Table 4.2). A summary of reported activating agents for thioglycosides 7, 36, 38, 54, 57, 59–91 is provided in Table 4.2. Possibilities for transforming thioglycosides to other types of glycosyl donors as described above, that makes chemoselective activation at the desired stage in a complex oligosaccharide synthesis possible, also open the way for orthogonal glycosylation strategies [92–94] to be applied to such synthesis.

By using ‘activating’ (by electron donation) groups for protecting the sugar moiety to be used as the glycosyl donor and by placing ‘deactivating’ (by electron withdrawal) groups as protecting groups on the acceptor sugar molecule it is

**Table 4.2**

Activators employed in glycosylations using alkyl/aryl thioglycosides as glycosyl donors

Catalyst/promoter	References
Hg(OAc) <sub>2</sub>	59
HgCl <sub>2</sub> /HgSO <sub>4</sub>	59,60
Hg(OBz) <sub>2</sub>	61
Cu(OTf) <sub>2</sub>	62
Hg(NO <sub>3</sub> ) <sub>2</sub>	38
Pd(ClO <sub>4</sub> ) <sub>2</sub> /Pb(ClO <sub>4</sub> ) <sub>2</sub>	63,64
NBS	54,65
PhHgOTf	66
MeOTf	67–69
CuBr <sub>2</sub> –Bu <sub>4</sub> NBr ± AgOTf	70
DMTST	71,72
NOBF <sub>4</sub>	36
MeSOTf	73
MeSBr	73
PhSeOTf	74
MeI	75
NIS–TfOH	76,77
IDCP	78
AgOTf/Hg(OTf) <sub>2</sub>	79
TBPA	80
NBS–TfOH	81
PhIO–Tf <sub>2</sub> O/acids	82,83
PhI(CF <sub>3</sub> CO <sub>2</sub> )	84
NBS–Ph <sub>2</sub> IOTf/LiClO <sub>4</sub>	85
I <sub>2</sub>	57
PhSOTf	86
NBP/NBA/NBB	87
I <sub>2</sub> –AgOTf	7
NIS–TMSOTf/AgOTf	88, 89
TrB(C <sub>6</sub> F <sub>5</sub> ) <sub>4</sub> –I <sub>2</sub> –DDQ	90
NBS–TMSOTf	91

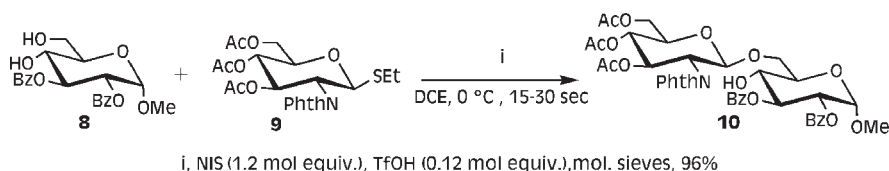
possible to use thioglycosides as both the glycosyl donor and the acceptor in a single reaction (for further details the reader is referred to Chapter 7). This approach was originally developed for 4-pentenyl glycoside coupling chemistry [77, 95, 96] and has been termed as the ‘armed–disarmed’ strategy [76, 78, 97, 98]. The objective of modulating the reactivity of glycosyl donor/acceptor molecules can also be achieved by changing the nature (by steric and electronic factors) of the alkyl/aryl substituent on the aglycon residue [4, 99–104]. The alkyl/aryl substituent on the aglycon moiety of the thioglycoside can also be chosen such that, after use in one reaction as a ‘disarmed’ glycosyl acceptor, it can easily be transformed into a more reactive species by a simple reaction, the so-called ‘latent-active’ strategy [105, 106]. Numerous examples are available from the literature that illustrate these strategies

as applied to syntheses ranging from simple monosaccharide glycosides to complex oligosaccharides. In the latter case examples of both 1,2-*cis*- and 1,2-*trans*-linkage formation can be found (for some recent examples see Refs. 107–113 and Crich and Smith, 2001). Thioglycosides have also been used in glycosylation reactions in which the acceptor residue is delivered intramolecularly [114–119] to the reaction site. Three representative methods chosen for presentation in this chapter include the use of (a) NIS–TfOH, (b) DMTST and (c) I<sub>2</sub>.

In addition to the solution-phase synthesis described here, thioglycosides have also been employed in solid-phase oligosaccharide synthesis [8]. Oligosaccharides can also be made from thioglycoside building blocks and the appropriate glycosyl acceptors *via* radical cation intermediates either by using tris(4-bromophenyl)ammoniumyl hexachloroantimonate [80] or by electrochemical means [120, 121].

#### Method 4

Preparation of methyl 2,3-di-*O*-benzoyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 6)-α-D-glucopyranoside (**10**) [78].



*Notes and discussion.* NIS–TfOH is a very strong activating agent for thioglycosides, both ‘armed’ and ‘disarmed’ thioglycosides can be successfully activated using this reagent system. In many cases the reaction reaches completion in short time periods. Although in the example described here DCE was used as solvent, other solvents such as neat DCM, MeCN, Et<sub>2</sub>O, toluene, etc. or a suitable mixed solvent system can also be used depending upon the temperature to be employed and the reaction desired, that is, whether 1,2-*cis*- or 1,2-*trans*-linked glycosides are to be prepared. Reactions can be carried out efficiently at temperatures ranging from –40 to 25 °C depending upon the nature of the reactants/products and the glycosidic linkage desired. Likewise, 3/4 Å molecular sieves are used more frequently than the 5 Å sieves described here. TfOH taken up in DCE or DCM or DCE–Et<sub>2</sub>O or DCM–Et<sub>2</sub>O mixture (TfOH is not very soluble in neat DCE or DCM) can often be added by titration by a syringe until the dark brown ‘iodine colour’ persists and the reaction is then monitored by TLC.

#### Materials.

Compound **8** [78]

irritant

Compound **9** (1.2 mol equiv.) [78]

irritant

Dry DCE, 1 ml/100 mg of <b>8</b> + <b>9</b>	irritant
NIS (1.2 mol equiv.)	irritant, light sensitive
Powdered molecular sieves, 5 Å, same weight as the sugar	irritant
TfOH, 1 M stock solution in	corrosive, irritant
DCE–Et <sub>2</sub> O, 1:1 (0.1 mol equiv.)	flammable
Celite <sup>®</sup>	irritant
CH <sub>2</sub> Cl <sub>2</sub>	irritant
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> , aq., 10%	irritant
Distilled water	
Sodium sulfate, granular, anhydrous	irritant

EtOAc/*n*-hexane (1:3–1:1) for chromatography and column chromatographic grade silica gel.

*Equipment.* The experiment must be conducted in a well-ventilated fume hood. As TfOH is extremely corrosive and reacts violently with water, direct contact with skin and water must be avoided. If a longer reaction time is indicated it may be advisable to protect the flask from light by wrapping with aluminium foil.

Round bottomed flask with magnetic stirring bar

Cooling bath

Magnetic stirrer

Syringe

Separatory funnel

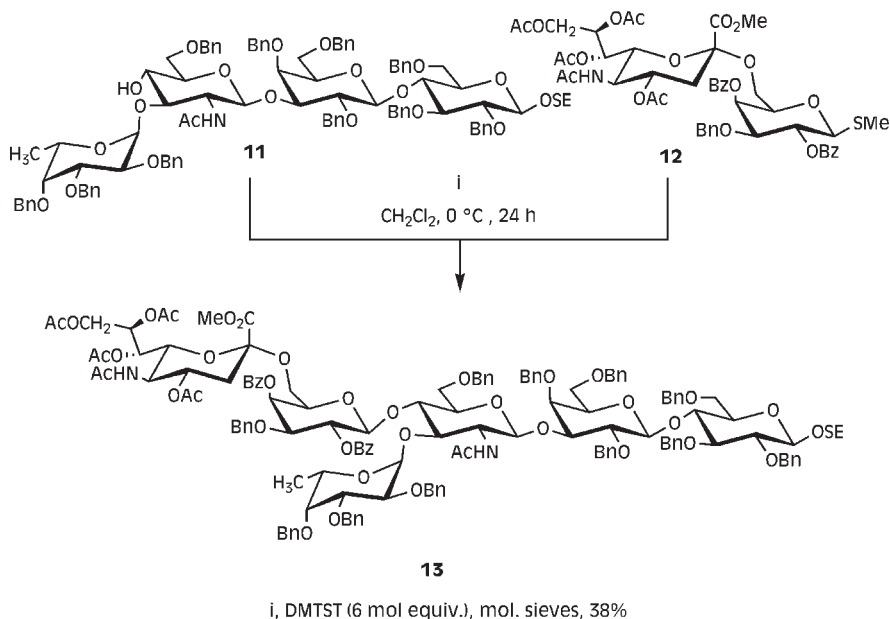
Rotary evaporator

Column chromatography equipment

*Procedure.* Add a solution of TfOH (0.1 mmol) in DCE/Et<sub>2</sub>O (1:1, 5 ml) to a stirred mixture of acceptor **8** (1 mmol), donor **9** (1.2 mmol), powdered molecular sieves, 5 Å (980 mg), NIS (1.2 mmol) and DCE (8 ml) held at 0 °C and continue stirring until the reaction is shown to be complete by TLC. (The title reaction was complete in 30 s.) Filter the reaction mixture through a bed of Celite<sup>®</sup> and wash the residue thoroughly with DCM. Subject the combined filtrate and washings to an aqueous work up by washing it successively with aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and water. After drying (anhydrous Na<sub>2</sub>SO<sub>4</sub>) concentrate the organic layer to a small volume and isolate the product by purification on a column of silica gel using EtOAc/*n*-hexane (1:3–1:1) as the eluent. The disaccharide (**10**) is obtained in 96% yield: [ $\alpha$ ]<sub>D</sub> = +78.2 (c 1.0, CHCl<sub>3</sub>); <sup>13</sup>C NMR (δCDCl<sub>3</sub>) 96.6 (C-1) and 99.0 (C-1').

*Method 5*

Preparation of hexasaccharide (**13**) [122].



*Notes and discussion.* A relatively difficult glycosylation reaction described in the literature is chosen as an example for illustrating the use of DMTST as a mild activating agent for thioglycosides. Although for several other less demanding cases good to excellent yields have been reported using DMTST, only a moderate yield has been recorded for the title preparation that involves a difficult  $\beta$ -(1  $\rightarrow$  4)-linkage formation. DMTST is not commercially available but can be prepared from an equimolar mixture of  $\text{Me}_2\text{S}_2$  and  $\text{MeOTf}$  in a trivial reaction (see below) and can be stored in a freezer for long periods. The reaction is best performed under anhydrous conditions.

Preparation of DMTST can be carried out as follows [123]. A solution of  $\text{MeOTf}$  in anhydrous DCM is treated with an equimolar quantity of  $\text{Me}_2\text{S}_2$  at rt until complete reaction occurred (1–2 d). Anhydrous ether is added to separate the product as crystals and is collected by filtration. They can be stored dry, preferably after mixing with an equal weight of activated molecular sieves (3 or 4 Å, powdered) in a freezer.

*Materials.*

Tetrasaccharide **11** [122]

irritant

Disaccharide **12** (1.5 mol equiv.) [122]

irritant

Dry CH <sub>2</sub> Cl <sub>2</sub> , 0.8–1 ml/100 mg of <b>11</b> + <b>12</b>	irritant
DMTST (4–6 mol equiv.)	corrosive, stench, irritant
Molecular sieves (powdered), 4 Å	irritant
MeOH	flammable
Triethylamine	irritant
Celite <sup>®</sup>	irritant
Distilled water	
Sodium sulfate, granular, anhydrous	irritant

EtOAc/*n*-hexane (4:1) for chromatography and column chromatographic grade silica gel.

*Equipment.* The experiment must be conducted in a well-ventilated fume hood. As DMTST is toxic and corrosive direct contact with skin must be avoided.

Round bottomed flask with magnetic stirring bar

Cooling bath

Magnetic stirrer

Syringe

Separatory funnel

Rotary evaporator

Column chromatography equipment

*Procedure.* Stir a mixture of acceptor **11** (0.3 mmol), donor **12** (0.48 mmol), powdered molecular sieves, 4 Å (1 g) and DCM (8 ml) for 8 h at rt. Cool the mixture to 0 °C and add, while being stirred, a mixture of DMTST (1.8 mmol) and powdered molecular sieves, 4 Å (450 mg) to it. Continue the stirring for 24 h at 0 °C during which time the reaction is monitored by TLC. Add MeOH (1 ml) and Et<sub>3</sub>N (0.5 ml) to the reaction mixture and separate the solids by filtration through a Celite<sup>®</sup>-pad. Wash the residue thoroughly with DCM, transfer the combined filtrate and washings to a separatory funnel and wash with water. After drying (anhydrous Na<sub>2</sub>SO<sub>4</sub>) the organic layer concentrate it to a small volume and isolate the product by purification on a column of silica gel (eluent: EtOAc/hexane, 4:1). The hexasaccharide **13** is obtained in 38% yield as an amorphous solid.  $[\alpha]_D = -13.0$  (*c* 0.83, CHCl<sub>3</sub>); <sup>1</sup>H NMR (δCDCl<sub>3</sub>) 0.99 (m, 2H, Me<sub>3</sub>SiCH<sub>2</sub>), 1.11 (d, 3 H, *J*<sub>5,6</sub> = 6.4 Hz, *H*-6, fucose unit), 1.6–2.07 (6s, 12H, 2 × NCOCH<sub>3</sub> and 4 × COCH<sub>3</sub>); 3.74 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 7.08–8.14 (m, 65H, Ar-*H*).

*Method 6*

Preparation of methyl (2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-galactopyranosyl)-(1  $\rightarrow$  3)-2,4,6-tri-*O*-acetyl-1-thio- $\beta$ -D-galactopyranoside (**16**) [57].



*i*, **14** (0.25 mmol), **15** (1.2 mol equiv.),  $\text{K}_2\text{CO}_3$  (1.2 mol equiv.),  $\text{I}_2$  (1.2 mol equiv.), mol. sieves, 71%

**Notes and discussion.** This example serves to illustrate the ‘armed–disarmed’ approach for disaccharide synthesis. Thus both the donor and the acceptor are thioglycosides and iodine, a relatively recent entry as a thioglycoside activator, is used to promote the reaction. In this reaction the acceptor thioglycoside is untouched by iodine because of the “deactivating” C-2-*O*-acetyl group present in the molecule. On the other hand the benzyl groups present in the donor thioglycoside render it susceptible to activation by iodine in an efficient manner. It is also striking that in this particular example the reaction proceeds stereospecifically giving the 1,2-*cis*-linked disaccharide in good yield. In other instances, however, mixtures of 1,2-*cis*- and 1,2-*trans*-linked products were obtained [57]. The solvent used for the reaction as well as the presence/absence of molecular sieves/acid scavenger can also affect the stereoselectivity [124]. The reactions are greatly robust and in many cases stringent anhydrous conditions or the presence of molecular sieves is not essential.

*Materials.*

Thioglycoside <b>14</b> [57]	irritant
Thioglycoside <b>15</b> (1.2 mol equiv.) [57]	irritant
Dry $\text{CH}_2\text{Cl}_2$ , approximately 1 ml/100 mg of <b>14</b> + <b>15</b>	irritant
Molecular sieves (powder), 3 Å	irritant
Iodine	irritant, vapour harmful
Potassium carbonate (1.2 mol equiv.)	irritant
Celite <sup>®</sup>	irritant
$\text{Na}_2\text{S}_2\text{O}_3$ , aq., 10%	irritant
Sodium sulfate, granular, anhydrous	irritant

EtOAc/*n*-hexane (1:3) for chromatography and column chromatographic grade silica gel.

*Equipment.* The experiment is best conducted in a well-ventilated fume hood. As iodine is corrosive direct contact with skin or inhalation of its vapour should be avoided.

Round bottomed flask with magnetic stirring bar  
Magnetic stirrer  
Syringe/pipette  
Separatory funnel  
Rotary evaporator  
Column chromatography equipment

*Procedure.* Add iodine (0.3 mmol) to a stirred mixture of acceptor **14** (0.25 mmol), donor **15** (0.3 mmol), powdered molecular sieves, 3 Å (200 mg),  $K_2CO_3$  (0.3 mmol) and DCM (3 ml) and continue stirring for 7 h at rt. Dilute the reaction mixture with DCM (10 ml) and separate the solids by filtration through a bed of Celite<sup>®</sup>. Wash the residue thoroughly with DCM, transfer the combined filtrate and washings to a separatory funnel and wash it with aq.  $Na_2S_2O_3$  solution. After drying (anhydrous  $Na_2SO_4$ ) the organic layer concentrate it to a small volume and isolate the product by purification on a column of silica gel (eluent: EtOAc/hexane, 1:3). The disaccharide **16** is obtained in 71% yield as an amorphous powder after lyophilisation from dioxane;  $^1H$  NMR ( $\delta_{CDCl_3}$ ) 1.86–2.19 (4s, 12H,  $3 \times OCOCH_3$  and  $SCH_3$ ); 4.23 (d, 1H,  $J_{1,2} = 9.9$  Hz,  $H-1$ ), 5.13 (d, 1H,  $J_{1',2'} = 3.4$  Hz,  $H-1'$ ), 7.31 (m, 20H, Ar-H).

## 4.3 SULFOXIDES

### 4.3.1 Preparation and activation

Although glycosyl sulfoxides (and sulfones) have long been known [49, 125–128], the use of glycosyl sulfoxides as glycosyl donors emanated primarily from the work of Kahne and co-workers [129–136]. Crich's group have applied glycosyl sulfoxides very successfully to the synthesis of  $\beta$ -mannosides [137–141]. Glycosyl sulfoxides are easily accessible from the corresponding thioglycosides by well-established methods that make use of either MCPBA [129] or hydrogen peroxide [142] as oxidising agent. We have found the latter method (described below) that uses hydrogen peroxide in the presence of silica at rt convenient and effective for preparing sulfoxides for glycosylation reactions. The MCPBA-method, as described by Crich and Sun [140], is also described below. In both methods sulfoxides are generally obtained as a diastereomeric mixture of *R* and *S* isomers (chiral at sulfur) and are used as such after isolation of the oxidation products by chromatography. Although in some cases exclusive formation of one or the other of the isomers is reported (see references below) any influence of their configuration on the products obtained after coupling with glycosyl acceptors has not been studied so far. While triflic anhydride is the most popular activating agent for sulfoxides [129, 130, 132–141, 143–146] other

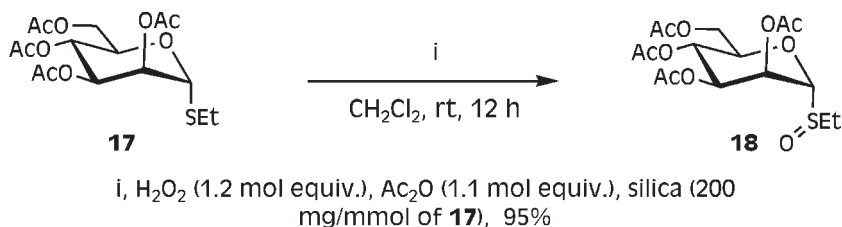


agents such as triflic acid [131], triflic acid–triethyl phosphite [147], trimethylsilyl triflate [100, 148] benzenesulfonyl triflate [139, 140] and iodine [124] have also been reported to be effective for the purpose. Methods using  $\text{Tf}_2\text{O}$  as the activating agent, as developed by Yan and Kahne [136], are described below in detail for the solution-phase preparation of a disaccharide (**21**). Application of sulfoxide chemistry to solid-phase oligosaccharide synthesis [135, 136] can be found else where in this book.

### 4.3.2 Procedures

#### Method 7

Preparation of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio- $\alpha$ -D-mannopyranoside *S*-oxide (**18**) [142].



*Notes and discussion.* Various protecting groups such as acetate esters, benzyl ethers and benzylidene acetals are compatible with this reaction and the yields are usually very good. The product is usually obtained as a mixture of *R* and *S* isomers and must either be used immediately or stored dry in the freezer.

#### Materials.

Ethyl tetra- <i>O</i> -acetyl-1-thio- $\alpha$ -D-mannopyranoside ( <b>17</b> )	irritant
$\text{CH}_2\text{Cl}_2$ , 1 ml/100 mg of <b>17</b>	irritant
Acetic anhydride, 1.1 mol equiv.	irritant
$\text{H}_2\text{O}_2$ , 35% solution, 1.2 mol equiv.	stench, irritant
$\text{NaHSO}_3$ , aq., saturated	irritant
$\text{NaHCO}_3$ , aq., saturated	irritant
Distilled water, saturated aq. brine	
Sodium sulfate, granular, anhydrous	irritant

EtOAc/*n*-hexane (1:4–3:5) for chromatography and column chromatographic grade silica gel.

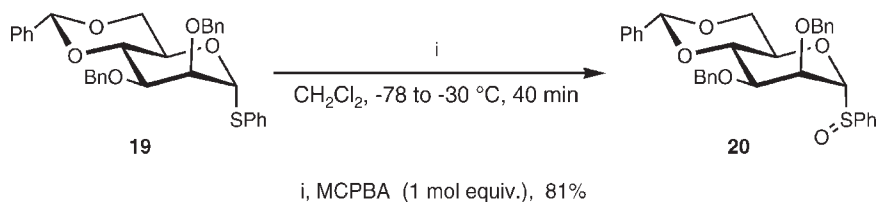
*Equipment.*

Round bottomed flask with magnetic stirring bar  
 Magnetic stirrer  
 Pipette or syringe  
 Separatory funnel  
 Rotary evaporator  
 Column chromatography equipment

*Procedure.* Add aq.  $\text{H}_2\text{O}_2$  (1.2 g, 35%, w/v solution) to a stirred mixture of **17** (3.9 g, 10 mmol), acetic anhydride (1.04 ml, 1.1 mol equiv.), silica gel (2 g, 220–440 mesh) and DCM (50 ml) and continue stirring for 12 h at rt. Dilute the reaction mixture with DCM and, after transferring it to a separatory funnel, wash successively with aq.  $\text{NaHSO}_3$  solution, aq.  $\text{NaHCO}_3$  solution and brine. Dry the organic layer (anhydrous  $\text{Na}_2\text{SO}_4$ ) and concentrate it to a small volume under reduced pressure. The residue on silica gel column chromatography (eluent: EtOAc/light petroleum, 1:4–3:5) yields **18** as a white solid (3.87 g, 95%). Mp 134–136 °C;  $^1\text{H}$  NMR ( $\delta\text{CDCl}_3$ ) 1.38 (t, 3H,  $\text{CH}_3\text{CH}_2\text{S}$ ), 2.02–2.18 (4s, 12H,  $4 \times \text{COCH}_3$ ); 4.66 (d, 1H,  $J_{1,2} = 2.0$  Hz,  $H-1$ );  $^{13}\text{C}$  NMR ( $\delta\text{CDCl}_3$ ) 90.6 (C-1).

*Method 8*

Preparation of phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio- $\alpha$ -D-mannopyranoside *S*-oxide (**20**) [140].



*Notes and discussion.* As in the preceding method various protecting groups such as acetate esters, benzyl ethers and benzylidene acetals are compatible with this reaction; the yields are also very good and the products are usually obtained as mixtures of *R* and *S* isomers. Washing with aqueous alkali is important in the aqueous work-up of the reaction mixture in order to ensure complete removal of the chlorobenzoic acid by-product.

*Materials.*

Phenyl thiomannoside derivative <b>19</b> [140]	irritant
$\text{CH}_2\text{Cl}_2$ , 4 ml/100 mg of <b>19</b> [140]	irritant
MCPBA, 60% solution, 1 mol equiv.	irritant

NaHCO<sub>3</sub>, aq., saturated

irritant

Saturated aq. brine

Sodium sulfate, granular, anhydrous

irritant

EtOAc/hexane (1:10–1:4) for chromatography and column chromatographic grade silica gel.

### Equipment.

Round bottomed flask with magnetic stirring bar

Magnetic stirrer

Cooling bath

Pipette or syringe

Separatory funnel

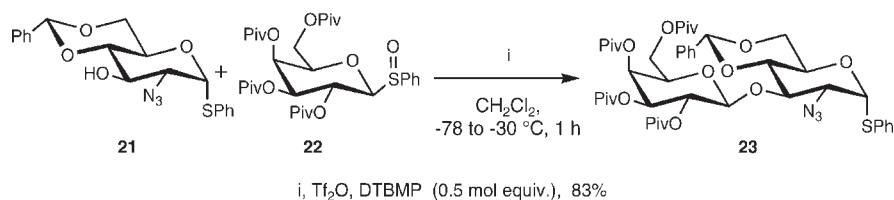
Rotary evaporator

Column chromatography equipment

**Procedure.** Add MCPBA (60%, 1.4 g, 5.55 mmol) to a stirred solution of **19** (3 g, 5.55 mmol) in DCM (50 ml) at  $-78^{\circ}\text{C}$ . While being stirred, allow the reaction mixture to warm up to  $-30^{\circ}\text{C}$  over 40 min. Add NaHCO<sub>3</sub> solution (to quench the reaction) and, after stirring, transfer the reaction mixture to a separatory funnel. Separate the organic layer, wash with brine, dry (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrate to a small volume under reduced pressure and purify by silica gel column chromatography (eluent: EtOAc/hexane, 1:10). The sulfoxide **20** is obtained as a solid (as a single, but unassigned isomer, 2.5 g, 81%). Mp  $136\text{--}139^{\circ}\text{C}$ ;  $[\alpha]_{\text{D}} = -52.7$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (δCDCl<sub>3</sub>) 4.51 (bs, 1H, *H*-1); 5.64 (s, 1H, PhCH); 7.21–7.55 (m, 15H, Ar-*H*); <sup>13</sup>C NMR (δCDCl<sub>3</sub>) 97.5 (C-1); 101.6 (PhCH).

### Method 9

Preparation of phenyl (2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (**23**) [136].



**Notes and discussion.** The coupling reaction should be performed under an inert gas atmosphere using anhydrous solvents. The entire operation should be carried out in a well-ventilated fume hood. Triflic anhydride is corrosive and must be handled with

care to avoid contact with skin; contact with water should also be avoided. Addition of the reactants and reagents must follow the order described in the procedure given below, particularly when donor molecules bearing non-participating C-2 substituents are being used for the glycosylation (e.g. for forming 1,2-*cis*-linkages), as this can affect the stereochemical outcome of the reaction [137]. Many acid and base sensitive protecting groups are tolerated [139, 140] and chemoselective activation of the sulfoxide centre, as seen in the present example, is achievable.

*Materials.*

Phenyl thioglycoside derivative <b>21</b> [136] (37 mg, 0.096 mmol)	irritant
Glycosyl sulfoxide derivative <b>22</b> [136] (109 mg, 0.175 mmol)	irritant
CH <sub>2</sub> Cl <sub>2</sub> , 5 ml	irritant
Tf <sub>2</sub> O, (15 µl, 0.089 mmol)	corrosive, irritant
DTBMP (110 mg, 0.526 mmol)	irritant
NaHCO <sub>3</sub> , aq., saturated	irritant
Sodium sulfate, granular, anhydrous	irritant

13% EtOAc in petroleum ether for chromatography and column chromatographic grade silica gel.

*Equipment.*

Round bottomed flask with magnetic stirring bar  
Magnetic stirrer  
Cooling bath  
Source of inert gas supply  
Syringe and rubber septa of appropriate size for the reaction flask  
Separatory funnel  
Rotary evaporator  
Column chromatography equipment

*Procedure.* Add Tf<sub>2</sub>O (15 ml, 0.089 mmol) to a stirred solution of sulfoxide **22** (0.175 mmol) in DCM (5 ml) taken in a round bottomed flask (under argon atmosphere) fitted with a rubber septum and held at –78 °C. Allow the mixture to warm up to –60 °C and stir at that temperature for 15 min. Add a solution of acceptor **21** (37 mg, 0.096 mmol) and DTBMP (10 mg, 0.526 mmol) in DCM (5 ml) dropwise to the reaction vessel *via* a syringe and continue stirring at –60 °C for an additional 10 min. Allow the reaction mixture to warm up to –30 °C over 30 min and subsequently quench the reaction by adding, while still being stirred, aq. NaHCO<sub>3</sub> solution (5 ml) to the flask. Transfer the mixture to a separatory funnel and wash the organic layer with aq. NaHCO<sub>3</sub> solution (2 × 100 ml) followed by drying (anhydrous Na<sub>2</sub>SO<sub>4</sub>) and concentration under reduced pressure to a small

volume. The residue on chromatography (eluent: 13% EtOAc in petroleum ether) yields (**23**) as a white solid (83%);  $^1\text{H}$  NMR ( $\delta\text{CDCl}_3$ ) 1.11, 1.14, 1.22, 1.23 (4s, 36H, 4  $\times$  Piv); 4.87 (d, 1H,  $J_{1',2'} = 8.1$  Hz,  $H-1'$ ); 5.55 (s, 1H, PhCH); 5.60 (d, 1H,  $J_{1,2} = 4.4$  Hz,  $H-1$ ); 7.2–7.5 (m, 10H, Ar-H);  $^{13}\text{C}$  NMR ( $\delta\text{CDCl}_3$ ) 87.3 (C-1); 100.5 (C-1'); 101.7 (PhCH); 176.6, 176.8, 177.3, 177.6 (4  $\times$  CO).

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## Synthesis and Activation of Carbohydrate Donors: Acetimidates, *n*-Pentenyl and Vinyl Glycosides

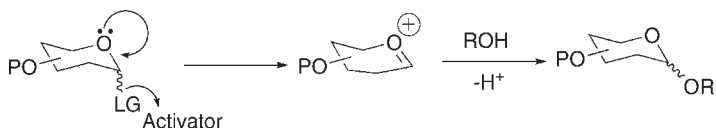
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Over the closing decades of the 20th century, detailed studies of biological systems revealed the prevalence and importance of oligosaccharides in natural processes. The synthesis of the glycon component of glycoconjugate molecules such as glycoproteins and glycolipids remains an area of intense research as we seek to further study the biological interactions involving such molecules. However, the sheer complexity of the oligosaccharides involved means that the synthetic strategies that must be employed must themselves be highly advanced, for the structural and functional diversity of natural carbohydrate derivatives is almost unending. There are three crucial problems that the chemist face when designing a strategy for an oligosaccharide synthesis [1, 2]. The first, that of regioselectivity, is usually solved, as it is elsewhere in chemical synthesis, by a carefully planned protecting group strategy—a technique which is in many ways more highly developed in carbohydrate chemistry than elsewhere. The second problem is that of the yield of the glycosylation step and a successful synthesis requires that the attachment of sugar units together must be an efficient process. For many years after the inception of carbohydrate chemistry by Emil Fischer at the end of the 19th century [3], and even after the development of improved glycosylation procedures such as the Koenigs–Knorr technique [4], glycosylation reactions were often difficult to carry out and/or low yielding. However, these problems have also been successfully addressed, as over the course of the latter part of the 20th century a number of methods for the highly efficient glycosylation of an aglycon alcohol (be it carbohydrate based or not) have emerged. The third and final problem in any given glycosylation step is that of anomeric stereoselectivity. Although this problem is yet

to be completely solved, in many cases a combination of suitable protecting group pattern and glycosylation conditions can in fact yield the desired anomer in a selective fashion.

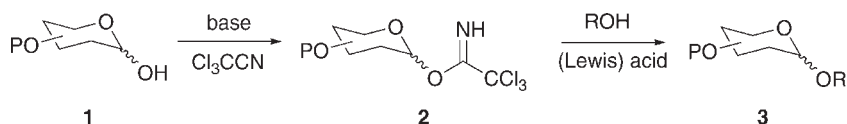


**Scheme 5.1**

The focus of this chapter is on three glycosylation methods that have found particular utility in oligosaccharide synthesis. Each method relies upon the preparation of a donor carbohydrate with specific anomeric functionality that is displaced selectively during the glycosylation reaction by the acceptor alcohol, often in an  $\text{S}_{\text{N}}1$ -like fashion via a glycosyl cation (Scheme 5.1). The three methods—anomeric acetimidates, anomeric *n*-pentenyl groups and vinyl glycosides—all involve the use of a donor sugar that has at its anomeric position a function which contains an unsaturated component. It is the reactivity of this component that allows the anomeric group to be displaced selectively during the glycosylation step. Naturally, each technique has distinct advantages and disadvantages and it is an unfortunate certainty in carbohydrate chemistry that no glycosylation technique finds utility in every situation, and the pros and cons of each method must be weighed before embarking on any synthesis involving a glycosylation step.

## 5.1 THE TRICHLOROACETIMIDATE METHOD

The successful use of imidates in glycosylation chemistry dates back to Sinaÿ's work, first published in 1976 and 1977 [5, 6]. However, it was Schmidt's trichloroacetimidate methodology, first communicated in 1980 [7], which really provided a powerful new glycosylation method for carbohydrate chemists. Since this first report, the trichloroacetimidate method has become a widely applicable procedure for glycoside bond formation, with hundreds of applications reported in the literature. The general outline for a glycosylation involving the trichloroacetimidate method is shown in Scheme 5.2. Starting from the hemi-acetal sugar **1**, the anomeric oxygen atom is derivatised with trichloroacetonitrile in a base-mediated reaction. Depending upon the conditions of this step, either the  $\alpha$ - or the  $\beta$ -anomer of the trichloroacetimidate **2** may be obtained. The anomeric group is then activated



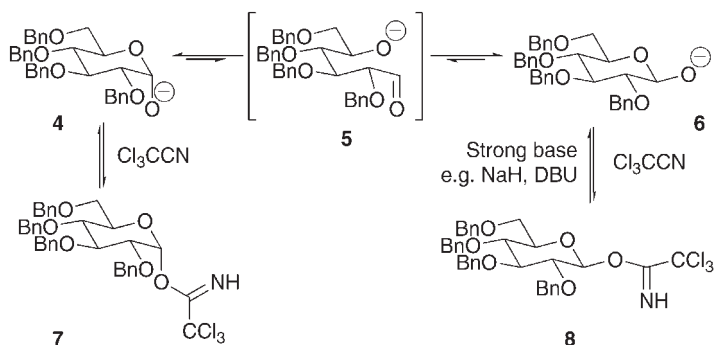
**Scheme 5.2**

during the glycosylation reaction with a mild Lewis acid such as boron trifluoride etherate resulting in loss of the imidate moiety and attack of the aglycon alcohol (ROH) to yield the desired glycoside **3**. The prime advantage of this technique is the high activity of the imidate under quite mild activating conditions. The use of trichloroacetimidates in glycosylation chemistry can be compared to nature's use of glycosyl phosphates as glycosyl donors, as indeed there are underlying mechanistic comparisons. It is not surprising then that this method of conducting glycosylation chemistry is one of the most frequently employed.

### 5.1.1 Preparation of trichloroacetimidates

The trichloroacetimide function is usually appended to the anomeric position immediately prior to glycosylation, as it is a reactive functional group that can be affected by previous steps in the synthesis. As noted above, anomeric trichloroacetimidates are prepared by functionalisation of a free anomeric hydroxyl group. However, in most cases, such a free hydroxyl group would interfere with other steps in a synthesis, and therefore it must be protected for all stages up until the trichloroacetimide is required. In general the hemi-acetal precursor is obtained through selective deprotection of a suitable anomeric protecting group. Since a number of protecting groups are capable of fulfilling this role this allows for some flexibility in the synthetic plan, negating somewhat the fact that the trichloroacetimide must generally be prepared directly prior to glycosylation. Of course, the fact that these extra manipulations are required for this approach may count against it if the glycosylation step in question is far along a synthetic pathway and the amount of available glycosyl donor is at a premium. Other candidates for glycosyl donors such as thioglycosides and *n*-pentenyl glycosides are inert to many of the standard manipulations in an oligosaccharide synthesis and these anomeric groups may be installed early in the synthetic sequence to be activated when required. Although many protecting groups could be employed to protect the anomeric hydroxyl group, there are a small number that are of particular utility in this role. Selective hydrolysis of an anomeric acetate group [8], activation of a *para*-methoxyphenyl group [9–11] or isomerization and subsequent hydrolysis of an anomeric allyl function [12] are all useful ways of accessing a free anomeric hydroxyl group. Enzymatic techniques may also be used to selectively deprotect an anomeric acetate *en route* to a trichloroacetimide [13]. The precise method of concealing the anomeric hydroxyl group depends largely on the other protecting groups present and the manipulations that must be applied to the glycoside before conversion to an acetimide. Once a free anomeric hemi-acetal has been made available, this may be treated with trichloroacetonitrile under basic conditions to furnish the desired anomeric acetimide. This procedure makes use of the fact that electron deficient nitriles undergo reversible base catalysed addition of alcohols to the carbon–nitrogen triple bond [14]. The fact that this process is reversible is important for the stereochemical outcome of the addition of the anomeric oxygen.

Depending upon the strength of the base used in the reaction it is possible to obtain either the  $\alpha$ - or  $\beta$ -anomer of the desired trichloroacetimidate. This is advantageous because the different anomers perform differently under conditions which promote  $S_N2$ -like glycosylation (*vide infra*). An entirely plausible mechanism to account for this observation has been proposed by Schmidt [15] (Scheme 5.3).



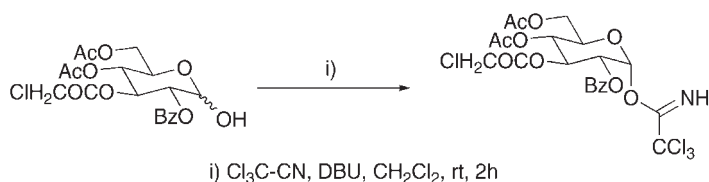
Scheme 5.3

The action of a base on a free anomeric hydroxyl group brings about base-catalysed *mutarotation* and thus the  $\alpha$ -anomer **4**, the open chain sugar **5**, and the  $\beta$ -anomer **6** interconvert as shown. It is safe to assume that the open chain alkoxide **5** is present in negligible amounts, as products from reaction of this form with electrophilic species are generally not observed. The  $\alpha$ - and  $\beta$ -anomers, however, do react with the electrophilic trichloroacetonitrile to yield imidates, although the equatorial  $\beta$ -anomer reacts more rapidly than the axial  $\alpha$ -anomer. This may be explained by way of the kinetic anomeric effect which states that the nucleophilicity of an equatorial anomeric alkoxide is enhanced through repulsions with the ring oxygen lone-pair orbitals. Taken in tandem with the fact that the steric constraints of the equatorial position are lower than that of the axial position, it is unsurprising that the kinetic product of the imidation reaction is the equatorial  $\beta$ -trichloroacetimidate, **8**. Assuming that a sufficiently weak base such as potassium carbonate is employed [16], the reverse reaction (deprotonation and subsequent elimination of the nitrile) is inhibited, and the rapid *mutarotation* ensures that the unreactive  $\alpha$ -alkoxide is able to convert into the  $\beta$ -alkoxide which subsequently reacts in an irreversible fashion to yield essentially pure  $\beta$ -trichloroacetimidate **8**. However, due to the *endo*-anomeric effect (of thermodynamic origin), the  $\alpha$ -trichloroacetimidate **4**, with an axial anomeric group, is the thermodynamic product of the reaction. If a stronger base such as sodium hydride [7, 17] or DBU [18] is used, this is able to bring about the reverse imidation reaction which the weaker base was unable to effect. Hence, the kinetic  $\beta$ -imide product is slowly converted back to the alkoxide and the nitrile. Over time, the thermodynamically favoured  $\alpha$ -product **7** is formed as the less reactive  $\alpha$ -alkoxide attacks the electrophile and the equilibrium is dragged towards the energetically favoured

product. Thus with careful selection of conditions, it is possible to obtain the desired trichloroacetimidate anomer exclusively. Other choices of strong base for  $\alpha$ -imide formation include caesium carbonate (possibly more useful for large scale preparations according to Urban *et al.* [19]) and potassium hydroxide under phase transfer conditions [20]. The stability of these anomeric trichloroacetimidates is much greater than the bromides that are often used in the classical Koenigs–Knorr procedure. Unlike the bromides, in general they are thermally stable and may be stored at low temperature for some time before use.

### Method 1

Formation of an  $\alpha$ -trichloroacetimidate: trichloroacetimidation of 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha/\beta$ -D-glucopyranose under thermodynamic control [21].



*Notes and discussion.* This procedure is used to form a thermodynamically favoured  $\alpha$ -trichloroacetimidate. It utilizes a relatively strong base (DBU) to maintain the equilibrium between  $\alpha$ - and  $\beta$ -imide products which leads to the eventual predominance of the thermodynamic product [18]. DBU is a popular alternative to sodium hydride, which may also be used to effect this transformation [17]. The  $\alpha$ -trichloroacetimidate product could be used under  $\text{S}_{\text{N}}2$ -like glycosylation conditions to provide access to  $\beta$ -glycosides [22, 23], although in this case the product has a participating group on *O*-2 which will dominate the stereocontrol of its glycosylation reactions.

### Materials.

4,6-Di- <i>O</i> -acetyl-2- <i>O</i> -benzoyl-3- <i>O</i> -chloroacetyl- $\alpha/\beta$ -D-glucopyranose [21] (4.5 g, 10.1 mmol)	irritant
Trichloroacetonitrile (3.0 cm <sup>3</sup> , 30 mmol)	toxic
DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) (0.2 cm <sup>3</sup> , 1.3 mmol)	causes burns
Anhydrous dichloromethane (100 cm <sup>3</sup> , distilled from calcium hydride)	risk of irreversible effects, toxic
Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful

*Equipment.*

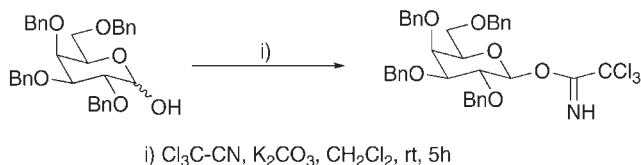
Round-bottomed flask (250 cm<sup>3</sup>) with rubber septum and magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Syringe (5 cm<sup>3</sup>) and needle  
Microsyringe (250  $\mu$ l) and needle  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha/\beta$ -D-glucopyranose (4.5 g, 10.1 mmol) by dissolving in anhydrous dichloromethane (100 cm<sup>3</sup>) under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Using a syringe, add trichloroacetonitrile (3.0 cm<sup>3</sup>, 30 mmol) and using a microsyringe add DBU (0.2 cm<sup>3</sup>, 1.3 mmol). After 2 h the reaction will be complete, as evidenced by TLC analysis (petroleum ether/ethyl acetate 3:1)—the  $\alpha$ -product should be visible as a major product spot (remembering that the  $\beta$ -imidate is also formed during the course of the reaction but is converted to the  $\alpha$ -compound). The reaction requires no work-up as removal of the solvent *in vacuo* affords the crude product which may be isolated in pure form by column chromatography using silica gel and petroleum ether/ethyl acetate 3:1, as the eluent. Although not strictly required in this specific procedure, it can be beneficial to add 1% triethylamine to the eluent in order to minimize decomposition of the product upon contact with acidic silica. The compound can be isolated as a colourless oil in 86% yield.  $[\alpha]_{\text{D}}^{20} = +103$  (*c* 1.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.09, 2.10 (2  $\times$  s, 2  $\times$  CH<sub>3</sub>CO, 6H), 3.94, 3.96 (ABq, *J* = 14.5 Hz, ClCH<sub>2</sub>CO, 2H), 4.35–4.17 (m, H-5, 2  $\times$  H-6, 3H), 5.34 (t, *J* = 10.0 Hz, H-4, 1H), 5.40 (dd, *J* = 3.5, 10.0 Hz, H-2, 1H), 5.84 (t, *J* = 10.0 Hz, H-3, 1H), 6.73 (d, *J* = 3.5 Hz, H-1, 1H), 7.59–7.41 (m, ArH, 3H), 7.97 (d, *J* = 7.0 Hz, ArH, 2H), 8.65 (s, NH, 1H). Anal. Calc. for C<sub>21</sub>H<sub>21</sub>Cl<sub>4</sub>NO<sub>10</sub>: C, 42.81; H, 3.59. Found: C, 42.93; H, 3.62%.

*Method 2*

Formation of a  $\beta$ -trichloroacetimidate: trichloroacetimidation of 2,3,4,6-tetra-*O*-benzyl- $\alpha/\beta$ -D-galactopyranose under kinetic control [24].



*Notes and discussion.* This procedure is used to form the kinetic  $\beta$ -trichloroacetimidate product in the imidation reaction and is based on the method reported by Schmidt *et al.* [16]. It utilizes a relatively mild base (potassium carbonate) which is essentially unable to catalyse the reverse imidation reaction. Hence equilibration between  $\alpha$ - and  $\beta$ -imide products is not possible and the kinetic product dominates. It is of note, however, that reactions of this type must be monitored carefully if the yield of the kinetic product is to be maximised. The  $\beta$ -trichloroacetimidate product could be used under  $\text{S}_{\text{N}}2$ -like glycosylation conditions to provide access to  $\alpha$ -glycosides [25].

*Materials.*

2,3,4,6-Tetra- <i>O</i> -benzyl- $\alpha/\beta$ -D-galactopyranose [24] (2.3 g, 4.2 mmol)	irritant
Trichloroacetonitrile ( $2.0 \text{ cm}^3$ , 20 mmol)	toxic
Potassium carbonate (2.3 g, 17.0 mmol)	harmful
Anhydrous dichloromethane ( $20 \text{ cm}^3$ , distilled from calcium hydride)	risk of irreversible effects, toxic
Celite <sup>®</sup>	harmful, possible risk of irreversible effects toxic
Dichloromethane ( $30 \text{ cm}^3$ )	risk of irreversible effects
Sodium bicarbonate (saturated aqueous solution)	assume toxic
Magnesium sulfate	assume toxic
Ethyl acetate, petroleum ether (bp 40–60 °C) and triethylamine for chromatography	highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful; highly flammable, harmful, causes severe burns



*Equipment.*

Round-bottomed flask (100 cm<sup>3</sup>) with rubber septum and magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Syringe (2 cm<sup>3</sup>) and needle  
Fritted glass funnel  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 2,3,4,6-tetra-*O*-benzyl- $\alpha/\beta$ -D-galactopyranose (2.3 g, 4.2 mmol) by dissolving in anhydrous dichloromethane (20 cm<sup>3</sup>) under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Using a syringe, add trichloroacetonitrile (2.0 cm<sup>3</sup>, 20 mmol) and then remove the septum and quickly add potassium carbonate (2.3 g, 17.0 mmol), reapplying the inert atmosphere after addition. After 5 h of vigorous stirring the reaction will be complete, as evidenced by TLC analysis (petroleum ether/ethyl acetate 7:3)—the  $\beta$ -product should be visible as a major product spot (remembering that small amounts of the  $\alpha$ -imide may also be formed during the course of the reaction). The reaction is worked-up by the addition of dichloromethane (30 cm<sup>3</sup>) and filtration through a bed of Celite<sup>®</sup> in a fritted glass funnel (about 2 cm high is sufficient; pre-pack the Celite<sup>®</sup> by running some dichloromethane through it). The solution should now be transferred into a separatory funnel and washed with saturated aqueous sodium bicarbonate solution (50 cm<sup>3</sup>). Drying the organic layers and removal of the solvent *in vacuo* affords the crude product which may be isolated in pure form by column chromatography using silica gel and petroleum ether/ethyl acetate 4:1, as the eluent. It is useful to add 1% triethylamine to the eluent in order to minimize decomposition of the product upon contact with acidic silica. The compound can be isolated as a syrupy oil in 80% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 3.63 (m, H-3, 2  $\times$  H-6, 3H), 3.73 (m, *J* = 6 Hz, H-5, 1H), 3.98 (t, *J* = 3.5 Hz, H-4, 1H), 4.09 (dd, *J* = 8.0, 9.5 Hz, H-2, 1H), 4.42 and 4.47 (ABq, *J* = 12.0 Hz, PhCH<sub>2</sub>, 2H), 4.73 (s, PhCH<sub>2</sub>, 2H), 4.81 and 4.90

(ABq,  $J = 10.5$  Hz,  $\text{PhCH}_2$ , 2H), 4.63 and 4.94 (ABq,  $J = 11.5$  Hz,  $\text{PhCH}_2$ , 2H), 5.75 (d,  $J = 8.0$  Hz, H-1, 1H), 7.25–7.34 (m, ArH, 20H), 8.62 (s, NH, 1H);  $^{13}\text{C}$  NMR (100.7 MHz,  $\text{CDCl}_3$ ) 68.1 (C-6), 73.0, 73.4, 74.8, 75.2 ( $4 \times \text{PhCH}_2$ ), 73.4 (C-4), 74.4 (C-5), 78.1 (C-2), 82.2 (C-3), 98.7 (C-1), 127.5, 127.6, 127.8, 127.9, 128.2, 128.4, 137.8, 138.2, 138.5 (Ar{C-1, C-2, C-3, C-4, C-5, C-6}), 161.5 (C=NH).

### 5.1.2 Glycosylation chemistry of trichloroacetimide donors

The power of the trichloroacetimide glycosylation method lies in the ability to activate the imide function into a good leaving group in a mild fashion. The driving force for this is the production of trichloroacetamide, which is liberated during the glycosylation reaction (Scheme 5.2). Coordination of a Lewis acid catalyst to the imide nitrogen atom promotes this process. However, the weak basicity of the amide nitrogen of trichloroacetamide, relative to that of the trichloroacetimide, means that once the amide is formed, the Lewis acid is liberated in favour of coordination to another imide. The result of this is that the Lewis acid acts in a catalytic fashion, which is a highly desirable feature of this method, as many other techniques (e.g. glycosylation of thioglycosides and the Koenigs–Knorr procedure) require multiple equivalents of the promoter species to bring about activation of the anomeric leaving group. Hence, the general procedure for conducting a trichloroacetimide glycosylation is to mix the donor and acceptor species in an inert solvent and add a catalytic amount of Lewis acid promoter. In some cases repeated additions of catalyst (or extended reaction times) are required, especially if orthoester intermediates are formed during the reaction which must then be isomerized into the desired glycosidic products. A number of different Lewis acidic promoters have been found to activate trichloroacetimidates, and the selection of an appropriate acid often depends largely upon the desired stereochemical result of the glycosylation, as well as being limited by other functionality present in both the donor and acceptor sugars. As well as boron trifluoride etherate, other useful promoters include trimethylsilyl trifluoromethanesulfonate (TMS triflate [26]), *tert*-butyldimethylsilyl triflate (TBDMS triflate [27]), triflic anhydride [28], zinc bromide [19], pyridinium *para*-toluenesulfonate (PPTS [29]), silver triflate (useful if boron trifluoride or TMS triflate promote rearrangement of protecting groups [30]) and dibutylboron triflate (useful if an unreactive acceptor alcohol is reacting with the promoter according to Wang *et al.* [31]). Some trichloroacetimide donors are more reactive in glycosylation reactions than others; specifically those with ester protecting groups tend to be less reactive than those that are ether-protected (see the section on armed and disarmed *n*-pentenyl glycosides and also Chapter 7 for more information). Very unreactive trichloroacetimide donors are prone to rearrangement in glycosylation reactions, becoming stable trichloroacetamides which detract from the yield of the process [32]. On the other hand, highly reactive donors are prone to decomposition prior to glycosylation, although this may be avoided in part by slowly adding the donor to a mixture of the acceptor and catalyst (the so called ‘inverse’ conditions [33, 34]). Finally, where unreactive

glycosyl acceptors are employed it is possible to obtain unexpected side reactions such as transfer of an anomeric thio group from the acceptor to the activated donor trichloroacetimidate [35].

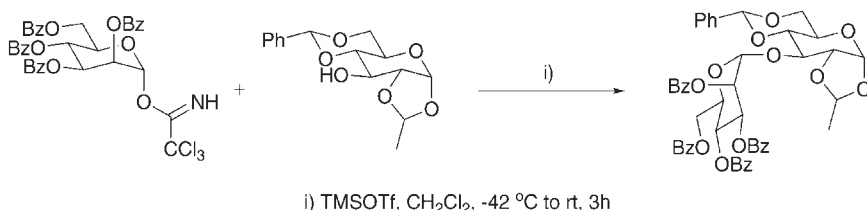
### 5.1.3 The stereochemical outcome of the glycosylation reaction

Predicting the anomeric stereochemistry of the product glycoside is a particularly complicated feature of glycosylation reactions that are not mediated by a well defined stereocontrolling element. However, it is very often possible to predict the stereochemical outcome of a trichloroacetimidate glycosylation, and therefore plan in advance for the desired result. Firstly, if the donor sugar contains a participating protecting group at the 2-position, such as 2-*O*-acyl, 2-*O*-benzoyl, 2-*N*-acyl or 2-*N*-phthaloyl, then the dominant factor in the glycosylation reaction will be this neighbouring group participation, and 1,2-*trans* glycosides will essentially be formed exclusively (giving e.g.  $\beta$ -*gluco* or  $\alpha$ -*manno*-type glycosides). However if the protecting group at the 2-position is non-participatory (e.g. a benzyl ether), then other factors can come into play and a mixture of anomers with a preponderance of the desired product may be the best achievable result. If low temperatures, non-polar solvents (e.g. dichloromethane) and, particularly, weak acid catalysts (such as boron trifluoride etherate) are employed, then trichloroacetimidate glycosylations mainly occur via an  $S_N2$ -type mechanism;  $\alpha$ -trichloroacetimidate donors give a predominance of  $\beta$ -products [22, 23] and  $\beta$ -trichloroacetimidates give a predominance of  $\alpha$ -products [25]. Therefore, the value of carefully selecting the anomeric configuration of the trichloroacetimidate donor in the preparation step becomes obvious. Conversely, if elevated temperatures, more polar solvents and/or a stronger acid catalyst (e.g. TMS triflate) are used then there is a greater likelihood of obtaining the thermodynamically and kinetically favoured product of attack on the glycosyl cation intermediate (i.e.  $\alpha$ -*gluco* or  $\alpha$ -*manno*-type glycosides). In this situation, the role of the solvent becomes more important. It has been plausibly proposed that the possibility of solvent participation at the anomeric position of the charged  $S_N1$ -type intermediate means that this may influence the eventual configuration of the glycosylated product. According to such proposals, an ethereal solvent coordinating to the anomeric position tends to lie in an equatorial position in accordance with the reverse anomeric effect (an effective positive charge lies on the ethereal oxygen atom). Such an ether presumably shields the  $\beta$ -face of the glycosyl cation, leading to attack from the  $\alpha$ -face and a predominance of  $\alpha$ -glycoside products [27]. It is also possible to promote the formation of  $\beta$ -glycosidic products with the use of acetonitrile as solvent [36]. It is proposed that if low temperatures are employed in the generation of the glycosyl cation, this may be attacked by acetonitrile along the kinetically favoured axial direction to form an  $\alpha$ -nitrilium ion [37]. Experiments suggest that conversion between this axially coordinated nitrile and the more stable equatorial intermediate (cf. the coordination of ethereal solvents) is relatively slow, and the acetonitrile may shield the  $\alpha$ -face, as

$\beta$ -glycosides can predominate. Whatever the actual role of the solvent in influencing the stereochemical outcome of the glycosylation reaction, the important point to note is that choice of solvent may be important. It should be borne in mind, however, that relying on selection of solvent to achieve the desired anomeric configuration is somewhat unreliable and that if a single anomer is required then it is far better to add further stereocontrolling elements to the synthetic plan (for example the use of a tethered intramolecular glycosylation; for a review see Ref. 38).

### Method 3

Neighbouring group participation in trichloroacetimidate glycosylations: glycosylation of 4,6-*O*-benzylidene-1,2-*O*-(*R/S*)-ethylidene- $\alpha$ -D-glucopyranose with 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-mannopyranosyl trichloroacetimidate [39].



*Notes and discussion.* This procedure is one of many examples in the literature where the stereocontrolling element of the glycosylation reaction is a 2-*O*-acyl group which participates by coordinating to C1 and therefore effectively blocks the *cis*-face of the glycosyl cation after it is formed. In this case, where the donor trichloroacetimidate is of the *manno*-type, the  $\beta$ -face of the glycosyl cation is blocked and the resulting *trans*-glycoside is an  $\alpha$ -mannoside. It is possible for orthoester intermediates to prevail in this type of glycosylation reaction, in which case these may be isomerized to the desired glycosides via the use of extended reaction times or repeated additions of the catalyst.

### Materials.

4,6- <i>O</i> -Benzylidene-1,2- <i>O</i> -( <i>R/S</i> )-ethylidene- $\alpha$ -D-glucopyranose [40] (0.293 g, 1 mmol)	irritant
2,3,4,6-Tetra- <i>O</i> -benzoyl- $\alpha$ -D-mannopyranosyl trichloroacetimidate [41] (0.741 g, 1 mmol)	irritant
Trimethylsilyl trifluoromethanesulfonate (TMS triflate) (20 $\mu$ l, 0.10 mmol)	causes burns, flammable
Anhydrous dichloromethane (60 cm <sup>3</sup> , distilled from calcium hydride)	risk of irreversible effects, toxic
Triethylamine (50 $\mu$ l)	highly flammable, harmful, causes severe burns

Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful
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*Equipment.*

High vacuum line  
Bunsen burner  
Round-bottomed flask (250 cm<sup>3</sup>) with a rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Cooling bath for –42 °C [42]  
Source of dry argon (or nitrogen) as inert gas  
Syringe and needle  
Microsyringe (50 µl) and needle  
Rotary evaporator  
Column chromatography equipment

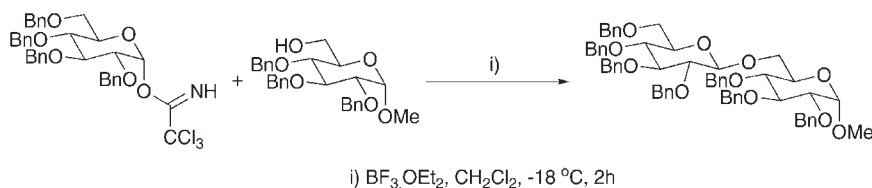
*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Glassware to be used under vacuum should be checked for star cracks before use. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. A –42 °C cooling bath may be prepared with the use of a solid carbon dioxide/acetonitrile mixture in a dewar, although a safer alternative is a carefully monitored mixture of solid carbon dioxide and isopropanol. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves. The two carbohydrate starting materials are exposed to high vacuum for 2 h before the reaction is carried out to ensure that they are moisture free.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 4,6-*O*-benzylidene-1,2-*O*-(*R/S*)-ethylidene- $\alpha$ -D-glucopyranose (0.293 g, 1 mmol) and 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-mannopyranosyl trichloroacetimidate (0.741 g, 1 mmol) to the flask by dissolving them in anhydrous dichloromethane under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum)—use a total of 60 cm<sup>3</sup> of dichloromethane. Cool this solution to –42 °C by immersing the flask in a cooling bath. Use a dry microsyringe to add TMS triflate (20 µl, 0.10 mmol) which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. After 3 h, during which the mixture is allowed to warm

to room temperature, the reaction will be complete, as evidenced by TLC analysis (petroleum ether/ethyl acetate 3:1). The reaction is worked-up by the syringe addition of triethylamine (50  $\mu$ l) to neutralise the mixture. Removal of the solvent *in vacuo* affords the crude product as a solid residue. The product may be isolated in pure form by column chromatography using silica gel and petroleum ether/ethyl acetate 3:1, as the eluent. This gives the compound as a colourless solid in 90% yield.  $[\alpha]_D = +13.0$  (*c* 1.1,  $\text{CHCl}_3$ ); mp 140–145  $^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.49 (d,  $\text{CH}_3\text{CH}$ , 3H), 3.74–3.83 (m, 2H), 3.97–4.00 (m, 1H), 4.16–4.20 (m, 2H), 4.40–4.50 (m, 2H), 4.67–4.72 (m, 2H), 5.21 (dd,  $\text{CH}_3\text{CH}$ ), 5.52 (d, H-1', 1H), 5.56 (d,  $J = 4.5$  Hz, H-1, 1H), 5.62 (s,  $\text{PhCH}$ , 1H), 5.84 (dd,  $J = 1.5$  Hz, H-2'), 5.95 (dd,  $J = 3.0$  Hz, H-3', 1H), 6.23 (t,  $J = 10.0$  Hz, H-4', 1H), 7.25–8.12 (m, ArH, 20H); Anal. Calc. for  $\text{C}_{49}\text{H}_{44}\text{O}_{15}$ : C, 67.43; H, 5.05. Found: C, 67.45; H, 5.03%.

#### Method 4

$\text{S}_{\text{N}}2$ -like trichloroacetimidate glycosylations: glycosylation of methyl 2,3,4-tri-*O*-benzyl- $\alpha$ -D-glucopyranoside with 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate [25].



**Notes and discussion.** This procedure is one of the first cited examples of the use of an  $\alpha$ -trichloroacetimidate donor undergoing  $\text{S}_{\text{N}}2$ -like displacement during a carefully controlled glycosylation reaction. The lack of a participating group on O-2 of the donor and the use of a non-participatory solvent, low temperature and mild activator are essential for the promotion of this process.

#### Materials.

Methyl 2,3,4-tri- <i>O</i> -benzyl- $\alpha$ -D-glucopyranoside [43] (0.558 g, 1.2 mmol)	irritant
2,3,4,6-Tetra- <i>O</i> -benzyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate [17] (1.10 g, 1.6 mmol)	irritant
Boron trifluoride diethyl etherate (3 $\text{cm}^3$ of a 0.4 M solution)	flammable, reacts violently with water liberating extremely flammable gases, causes burns
Anhydrous dichloromethane (20 $\text{cm}^3$ , distilled from calcium hydride)	risk of irreversible effects, toxic

Sodium carbonate	irritant
Sodium bicarbonate (saturated aqueous solution)	assume toxic
Sodium sulfate	irritant
Chloroform, diethyl ether and petroleum ether (bp 40–60 °C) for chromatography	harmful, irritant, possible risk of irreversible effects; highly flammable, harmful, may cause drowsiness or dizziness; highly flammable, harmful

#### *Equipment.*

Bunsen burner  
Round-bottomed flask (100 cm<sup>3</sup>) with a rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Cooling bath for – 18 °C [42]  
Source of dry argon (or nitrogen) as inert gas  
Syringe (3 cm<sup>3</sup>) and needle  
Separatory funnel (250 cm<sup>3</sup>)  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. A – 18 °C cooling bath may be prepared with the use of a liquid nitrogen/*ortho*-dichlorobenzene mixture in a dewar, although a safer alternative is a carefully monitored mixture of solid carbon dioxide and isopropanol. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

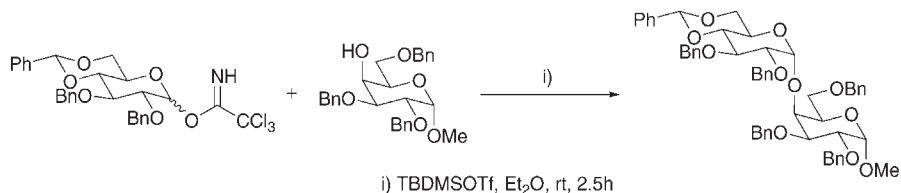
*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add methyl 2,3,4-tri-*O*-benzyl- $\alpha$ -D-glucopyranoside (0.558 g, 1.2 mmol) and 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate (1.10 g, 1.6 mmol) to the flask by dissolving them in anhydrous dichloromethane under argon and adding to the reaction vessel via cannula under inert gas pressure

(an exit needle or bubbler will be required in the receiving flask's septum)—use a total of 20 cm<sup>3</sup> of dichloromethane. Cool this solution to  $-18^{\circ}\text{C}$  by immersing the flask in a cooling bath. Use a dry syringe to slowly add boron trifluoride etherate (3 cm<sup>3</sup> of a 0.4 M solution) over the course of 45 min. This must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. After 2 h at  $18^{\circ}\text{C}$  the reaction will be complete, as evidenced by TLC analysis (chloroform/diethyl ether 20:1). The reaction is worked-up by allowing it to warm to room temperature, then adding excess solid sodium carbonate to neutralise the mixture. After allowing this to stir, add a mixture of dichloromethane (50 cm<sup>3</sup>) and saturated aqueous sodium bicarbonate solution (50 cm<sup>3</sup>) portionwise and transfer the whole solution into a separatory funnel. Drain off the lower organic layer and dry this with sodium sulfate. Removal of the solvent *in vacuo* affords the crude reaction mixture as an oily residue which may be filtered through a column of silica gel (using chloroform/diethyl ether 20:1 as the eluent) to provide the product as a mixture of  $\alpha$ - and  $\beta$ -anomers in 90% yield. The anomers may be separated by column chromatography using silica gel and chloroform/petroleum ether/diethyl ether 20:8:1, as the eluent. This gives the  $\alpha$ -anomer as a syrupy oil in 5% yield and the  $\beta$ -anomer as colourless crystals in 85% yield.  $\alpha$ -Anomer:  $[\alpha]_{\text{D}}^{20} = +57$  (*c* 1.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR [44] (500 MHz, CDCl<sub>3</sub>) 3.35 (s, OCH<sub>3</sub>, 3H), 3.44 (dd, *J* = 9.5, 3.5 Hz, 1H), 3.54 (dd, *J* = 9.5, 3.5 Hz, 1H), 3.55 (dd, *J* = 10.5, 2.0 Hz, 1H), 3.65 (m, 3H), 3.71 (dd, *J* = 11.5, 2.5 Hz, 1H), 3.79 (m, 3H), 3.95 (t, *J* = 9.5 Hz, 1H), 3.98 (t, *J* = 9.5 Hz, 1H), 4.45 (d, *J* = 11.0 Hz, PhCH<sub>2</sub>, 1H), 4.42 (d, *J* = 12.0 Hz, PhCH, 1H), 4.55 (d, *J* = 3.5 Hz, H1', 1H), 4.57 (d, *J* = 12.0 Hz, PhCH, 1H), 4.57 (d, *J* = 12.0 Hz, PhCH, 1H), 4.64 (d, *J* = 11.5 Hz, PhCH, 1H), 4.66 (s, PhCH<sub>2</sub>, 2H), 4.71 (d, *J* = 12.0 Hz, PhCH, 1H), 4.77 (d, *J* = 11.0 Hz, PhCH, 1H), 4.81 (d, *J* = 11.0 Hz, PhCH, 1H), 4.82 (d, *J* = 11.0 Hz, PhCH, 1H), 4.91 (d, *J* = 11.0 Hz, PhCH, 1H), 4.94 (d, *J* = 11.0 Hz, PhCH, 1H), 4.96 (d, *J* = 11.0 Hz, PhCH, 1H), 4.98 (d, *J* = 3.5 Hz, H1, 1H), 7.1–7.4 (m, ArH, 35H);  $\beta$ -anomer:  $[\alpha]_{\text{D}}^{20} = +17.9$  (*c* 1, CHCl<sub>3</sub>); mp 133–134 °C (from chloroform/ethanol); <sup>1</sup>H NMR [44] (500 MHz, CDCl<sub>3</sub>) 3.32 (s, OCH<sub>3</sub>, 3H), 3.42 (m, 1H), 3.51 (m, 3H), 3.56 (t, *J* = 9.5 Hz, 1H), 3.62 (t, *J* = 9.0 Hz, 1H), 3.67 (dd, *J* = 11.0, 5.0 Hz, 1H), 3.72 (dd, *J* = 11.0, 1.5 Hz, 1H), 3.82 (m, 1H), 3.98 (t, 1H, *J* = 9.5 Hz, 1H), 4.18 (dd, *J* = 10.5, 2.0 Hz, 1H), 4.34 (d, *J* = 8.0 Hz, H1, 1H), 4.52 (m, 3H), 4.58 (d, *J* = 11.0 Hz, PhCH, 1H), 4.60 (d, *J* = 3.5 Hz, H-1'), 4.65 (d, *J* = 12.0 Hz, PhCH, 1H), 4.71 (d, *J* = 11.0 Hz, PhCH, 1H), 4.74 (d, *J* = 11.0 Hz, PhCH, 1H), 4.79 (m, 4 × PhCH, 4H), 4.90 (d, *J* = 11.0 Hz, PhCH, 1H), 4.96 (d, *J* = 10.5 Hz, PhCH, 1H), 4.97 (d, *J* = 11.0 Hz, PhCH, 1H), 7.1–7.4 (m, ArH, 35H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) 55.1 (OCH<sub>3</sub>), 66.0, 68.4, 70.2, 70.3, 72.3, 73.4, 74.9, 75.0, 75.5, 75.7, 77.5, 77.7, 79.9, 80.1, 81.6, 82.1 (C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6', 7 × PhCH<sub>2</sub>) 97.2, 97.9 (C-1, C-1'), 127.5, 127.6, 127.7, 127.7, 127.8, 127.8, 128.0, 128.2, 128.3, 128.4, 137.9, 138.1, 138.4, 138.4, 138.8 (Ar{C-1, C-2, C-3, C-4, C-5, C-6}); MS *m/z* (FAB) C<sub>62</sub>H<sub>65</sub>O<sub>11</sub> (MH) requires 985.4527, found 985.4531.



### Method 5

Stereocontrol by solvent participation in trichloroacetimidate glycosylations: glycosylation of methyl 2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranoside with 2,3-di-*O*-benzyl-4,6-*O*-benzylidene- $\alpha/\beta$ -D-glucopyranosyl trichloroacetimidate [45].



**Notes and discussion.** This procedure illustrates the use of diethyl ether to achieve solvent mediated stereocontrol during a trichloroacetimidate glycosylation, in this case to provide a predominance of the  $\alpha$ -glycosidic product [27]. Use of acetonitrile on the other hand, generally gives a predominance of the  $\beta$ -product [36]. Note the absence of a participating protecting group on *O*-2, which would otherwise dictate the stereochemical outcome of the glycosylation reaction. It is also noteworthy that a specific anomer of the trichloroacetimidate donor is not isolated for use in this reaction; the configuration of the starting sugar is irrelevant when the glycosylation proceeds via an S<sub>N</sub>1-like process.

### Materials.

Methyl 2,3,6-tri- <i>O</i> -benzyl- $\alpha$ -D-galactopyranoside [46] (1.34 g, 2.88 mmol)	irritant
2,3-Di- <i>O</i> -benzyl-4,6- <i>O</i> -benzylidene- $\alpha/\beta$ -D-glucopyranosyl trichloroacetimidate [45] (2.76 g, 4.65 mmol)	irritant
<i>tert</i> -Butyldimethylsilyl trifluoromethanesulfonate (TBDMS triflate) (0.355 cm <sup>3</sup> , 1.55 mmol)	flammable, causes burns
Anhydrous diethyl ether (63 cm <sup>3</sup> , distilled from sodium/benzophenone)	highly flammable, harmful, may cause drowsiness or dizziness
Sodium bicarbonate	assume toxic
Sodium bicarbonate (saturated aqueous solution)	assume toxic
Diethyl ether	highly flammable, harmful, may cause drowsiness or dizziness
Sodium sulfate	irritant
Dichloromethane, ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	risk of irreversible effects; highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful

*Equipment.*

Bunsen burner  
Round-bottomed flask (250 cm<sup>3</sup>) with a rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Microsyringe (0.5 cm<sup>3</sup>)  
Separatory funnel (250 cm<sup>3</sup>)  
Rotary evaporator  
Column chromatography equipment

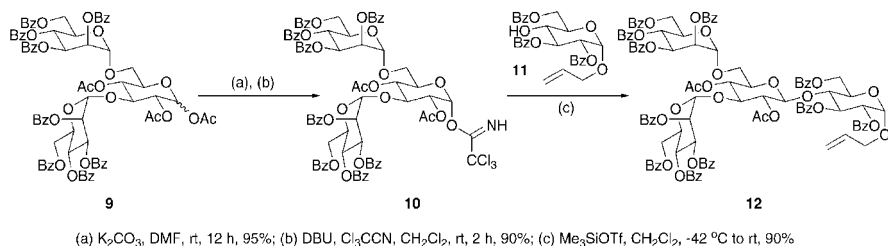
*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. A –18 °C cooling bath may be prepared with the use of a liquid nitrogen/*ortho*-dichlorobenzene mixture in a dewar, although a safer alternative is a carefully monitored mixture of solid carbon dioxide and isopropanol. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add methyl 2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranoside (1.34 g, 2.88 mmol) and 2,3-di-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ / $\beta$ -D-glucopyranosyl trichloroacetimidate (2.76 g, 4.65 mmol) to the flask by dissolving them in anhydrous diethyl ether under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum)—use a total of 63 cm<sup>3</sup> of dichloromethane. Use a dry microsyringe to add TBDMS triflate (0.355 cm<sup>3</sup>, 1.55 mmol) which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. After stirring for 2.5 h the reaction will be complete, as evidenced by TLC analysis (dichloromethane/petroleum ether/ethyl acetate 40:30:1). The reaction is worked-up by adding excess solid sodium bicarbonate to neutralise the mixture. The whole solution should now be transferred into a separatory funnel and then washed with saturated aqueous sodium bicarbonate (65 cm<sup>3</sup>). After separation of the organic layer, the aqueous layer should be re-extracted with diethyl ether (3  $\times$  75 cm<sup>3</sup>) and these extracts should be combined with the original organic layer. Drying the organic layers with sodium sulfate and removal of the solvent *in vacuo* affords the crude reaction mixture as an oil. The desired product may be separated by column chromatography using silica gel and dichloromethane/petroleum ether/ethyl acetate 40:30:1 to 40:30:4, as

the eluent. This gives the  $\alpha$ -anomer as an oil in 90% yield and the  $\beta$ -anomer as an oil in 9% yield (data not listed).  $\alpha$ -Anomer:  $[\alpha]_D^{28} = +49.6$  (*c* 2.7,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) 3.36 (s,  $\text{OCH}_3$ , 3H), 3.46–3.53 (m, H-3, H-2, H-2', 3H), 3.57 (dd,  $J = 3.5$  Hz, H-6, 1H), 3.61 (t,  $J = 9.5$  Hz, H-4', 1H), 3.81 (dd,  $J = 5.0$  Hz, H-6, 1H), 3.82–3.92 (m, H-5, H-5', 2H), 3.95 (dd,  $J = 3.5, 10.5$  Hz, H-6, 1H), 4.01 (t,  $J = 9.5$  Hz, H-3', 1H), 4.07 (m, H-4, 1H), 4.25 (non-first order ABq,  $\text{PhCH}_2$ , 2H), 4.32 (dd,  $J = 5.0, 10.0$  Hz, H-6'), 4.69 (d,  $J = 3.5$  Hz, H-1, 1H), 4.69 (d,  $J = 12.0$  Hz,  $\text{PhCH}$ , 1H), 4.76 (non-first order ABq,  $\text{PhCH}_2$ , 2H), 4.79 (s,  $\text{PhCH}_2$ , 2H), 4.87 (d,  $J = 12.0$  Hz,  $\text{PhCH}$ , 1H), 4.88 (d,  $J = 12.0$  Hz,  $\text{PhCH}$ , 1H), 4.94 (d,  $J = 4.0$  Hz, H-1', 1H), 4.95 (d,  $J = 10.5$  Hz,  $\text{PhCH}$ , 1H), 5.52 (s,  $\text{PhCHO}_2$ , 1H), 7.17–7.37 (m, ArH, 30H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ) 55.3 ( $\text{OCH}_3$ ), 63.0, 68.0, 68.9, 69.2, 72.8, 72.9, 73.4, 74.2, 74.3, 75.1, 77.2, 77.7, 79.1, 79.6, 82.8 (C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6',  $5 \times \text{PhCH}_2$ ), 99.0, 100.4, 101.0 (C-1, C-1',  $\text{PhCHO}_2$ ), 126.0, 127.3, 127.5, 127.5, 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.3, 128.4, 128.4, 128.7 (Ar{C-2, C-3, C-4, C-5, C-6}), 137.7, 138.2, 138.3, 138.5, 138.8, (Ar{C-1}); Anal. Calc. for  $\text{C}_{55}\text{H}_{58}\text{O}_{11}$ : C, 73.81; H, 6.53. Found: C, 73.73; H, 6.54%.

### 5.1.4 Glycosylation reactions with trichloroacetimidates: summary

A representative example of the use of the trichloroacetimidate technique in oligosaccharide synthesis is shown in Scheme 5.4, which illustrates the latter stages of a synthesis of a tetrasaccharide by Zhu and Kong [39]. Thus, trisaccharide **9**, which has itself been prepared via two previous trichloroacetimidate-mediated glycosylation steps, is selectively deacetylated with potassium carbonate and then converted into the trichloroacetimidate **10** in 86% overall yield. Reaction with acceptor sugar **11** under standard trichloroacetimidate coupling conditions provides tetrasaccharide **12** in 90% yield. In the reported synthesis, this oligosaccharide was subsequently deacylated, but in theory, the anomeric allyl group of **12** could be selectively hydrolysed and a further trichloroacetimidate coupling could be attempted. Thus this synthesis illustrates the main features of the trichloroacetimidate method in that the imidate moiety must be prepared directly before the glycosylation step, but assuming this is feasible, the glycosylation can often be

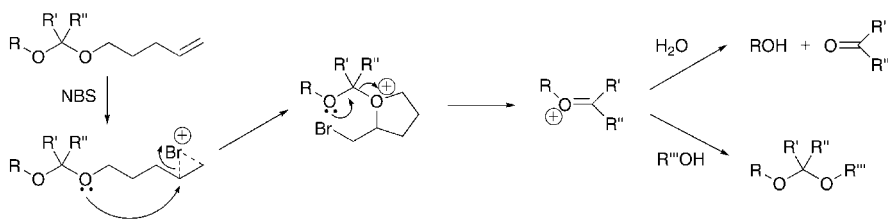


Scheme 5.4

carried out under mild and high yielding conditions. This explains why this method of glycosylation chemistry is one of the most often employed in oligosaccharide synthesis.

## 5.2 USE OF *n*-PENTENYL GLYCOSIDES AS GLYCOSYL DONORS

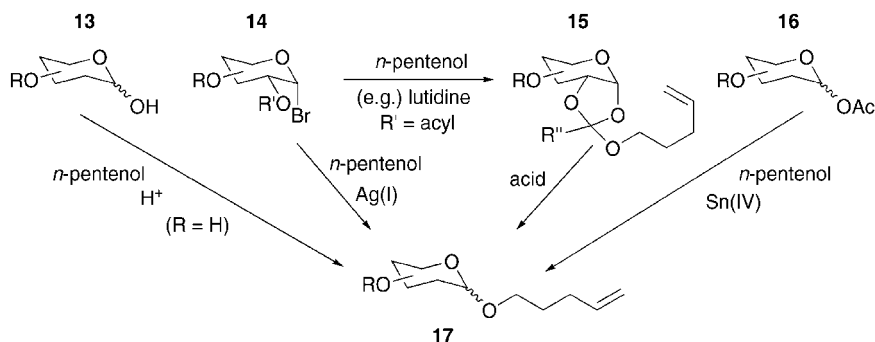
The study of the glycosylation chemistry of *n*-pentenyl glycosides (NPGs) has provided carbohydrate chemists with very helpful insights into the mechanism and reactivity of glycosyl donors in general, as well as a useful method for constructing large oligosaccharide targets. It is of interest to note therefore, that NPGs were discovered essentially more by chance than by design, during Fraser-Reid's work on the synthesis of part of streptovaricin A [47]. However, chance discovery or not, Fraser-Reid quickly applied the chemistry of the oxidative hydrolysis of *n*-pentenyl acetals with *N*-bromosuccinimide (NBS) (Scheme 5.5) [48] to develop firstly the *n*-pentenyloxymethyl-based protecting group [49] and secondly *n*-pentenyl-based glycosides. The original intention was to use the *n*-pentenyl group as a protecting group for the anomeric position, which could be liberated under mild conditions (NBS/water) to reveal the anomeric hydroxyl group, conditions which would not act upon other acetal protecting groups such as benzylidene or acetonide acetals [50, 51]. Whilst NPGs were successful in this function, it was noted that if the water in the hydrolysis reaction were replaced with an alcohol, then such an alcohol could efficiently capture the anomeric position. Thus the glycosylation chemistry of NPGs was born [52].



Scheme 5.5

### 5.2.1 Preparation of *n*-pentenyl glycosides

When employed as an anomeric leaving group, the *n*-pentenyl function is usually installed at the outset of the synthetic sequence, as it is far easier to add it at this point than at a later stage. Since the *n*-pentenyl group is inert to many of the standard manipulations that are carried out during an oligosaccharide synthesis, this can be an advantage in contrast to other glycosyl donors such as glycosyl bromides or trichloroacetimidates. There are a number of ways in which the *n*-pentenyl group can be added to the anomeric position (Scheme 5.6).



Scheme 5.6

Standard Fischer glycosylation of the unprotected sugar with *n*-pentanol under acidic conditions works well for glucose, mannose [53] and fucose [54] (e.g. **13**  $\rightarrow$  **17**). The commercially available but expensive *n*-pentanol may be successfully recovered after this procedure by distillation and saved for subsequent applications. Koenigs–Knorr glycosylation of suitable glycosyl bromides with, for example, silver triflate (suitable for perbenzoylated glycosyl bromides [47]) or silver carbonate (for peracetylated bromides [55]) also provides access to NPGs (e.g. **14**  $\rightarrow$  **17**). This method is more suitable for galactose and glucosamine, for which the Fischer technique is less useful. Glycosylation of glycosyl acetates, using tin(IV) chloride as a promoter for example, provides a third, if less often utilised avenue for entry into NPGs [47] (e.g. **16**  $\rightarrow$  **17**). Finally, it is interesting to note that NPGs may also be prepared via acid catalysed rearrangement of *n*-pentenyl orthoesters (e.g. **15**  $\rightarrow$  **17**). The orthoesters themselves are prepared under basic conditions from 2-*O*-acyl protected glycosyl bromides and *n*-pentanol [47]. Base mediated manipulations of the other positions of the sugar whilst the 1,2-orthoester is in place may then be carried out, before rearrangement to afford an NPG, which leaves a 2-*O*-acyl group in place—this strategy can provide selective access to the 2-position [56]. The NPGs themselves are often crystalline and may be stored at room temperature for long periods of time without degradation. The methods of preparation described above can often yield a mixture of  $\alpha$ - and  $\beta$ -anomers. For example, Fischer glycosylation of glucose with *n*-pentanol yields a thermodynamic 2:1 mixture of  $\alpha$ - and  $\beta$ -glucosides [53]. However, in cases where this is thought to be a problem, the  $\alpha$ - and  $\beta$ -anomers can often be separated by fractional crystallisation or column chromatography. Since this separation would generally take place at the start of the synthetic sequence, loss of material through anomeric mixtures is of less consequence than it would be in later stages. Work by Ruiz and co-workers [57] has shown that a single anomer of an NPG may also be obtained by selective hydrolysis of the anomer which is not required using a glycosidase. This leads to a high recovery of starting material that might otherwise be wasted.

*Method 6*

Preparation of *n*-pentenyl glycosides: pent-4-enyl  $\alpha/\beta$ -D-glucopyranoside from D-glucose [53].



i) *n*-pentenol, TfOH, DMSO, 90 °C, 2 days

*Notes and discussion.* Fischer glycosylation of a reducing sugar provides an anomeric mixture of *n*-pentenyl glycosides and is a straightforward method for obtaining such materials. Separation of the anomers produced through this technique is non-trivial, although this is usually of little consequence since glycosylation reactions of NPGs are insensitive to anomeric configuration and mixtures of anomers can be employed. Like all Fischer glycosylations, significant amounts of furanosides are formed initially, and these must be allowed to convert to the desired pyranoside form before the reaction is quenched. However, if the reaction is allowed to continue for too long, it becomes very discoloured and an intractable mixture is the result—careful monitoring is therefore essential.

*Materials.*

D-Glucose (5 g, 27.8 mmol)	assume toxic
4-Penten-1-ol (8.6 cm <sup>3</sup> , 83.4 mmol)	assume toxic
Dimethylsulfoxide (25 cm <sup>3</sup> )	irritant
Trifluoromethanesulfonic acid (triflic acid, 0.5 cm <sup>3</sup> , 5.7 mmol)	highly flammable, harmful
Activated carbon (charcoal)	harmful, irritant
Triethylamine	highly flammable, harmful, causes severe burns
Methanol and dichloromethane for chromatography	highly flammable, toxic, toxic: danger of very serious irreversible effects; risk of irreversible effects

*Equipment.*

Round-bottomed flask (100 cm<sup>3</sup>) with water condenser and magnetic stirrer bar  
 Magnetic hotplate stirrer with silicon oil heating bath and temperature probe  
 Syringe (10 cm<sup>3</sup>) and needle

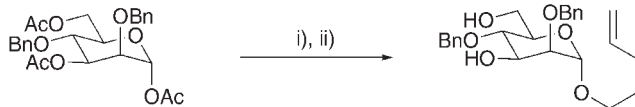
Microsyringe (0.5 cm<sup>3</sup>) and needle  
 Equipment for vacuum distillation  
 Column chromatography equipment

*Special precautions.* Glassware to be used under vacuum should be checked for star cracks before use. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a water condenser. Add D-glucose (5 g, 27.8 mmol), dimethylsulfoxide (25 cm<sup>3</sup>) and 4-penten-1-ol (8.6 cm<sup>3</sup>, 83.4 mmol). Use a microsyringe to add triflic acid (0.5 cm<sup>3</sup>, 5.7 mmol) which should fume during use and should be handled with care. After heating to 90 °C and stirring for 2 days the reaction will be complete, as evidenced by TLC analysis (methanol/dichloromethane 3:17). The reaction is worked-up by decolourising with activated carbon, then filtration and addition of triethylamine (0.5 cm<sup>3</sup>). The pentenol and dimethylsulfoxide can then be collected by vacuum distillation—this mixture may be reused for subsequent Fischer glycosylations. The residue is the crude product which may be purified by column chromatography using silica gel and methanol/dichloromethane 3:17, as the eluent. This gives the desired product as an oil in 56% yield, an inseparable mixture of  $\alpha$ - and  $\beta$ -anomers, along with some recovered starting material. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 4.65 (br s, H-1 $\alpha$ , 1H), 4.80 (m, CH<sub>2</sub>CH=CH<sub>2</sub> (both anomers overlapping), 2H), 5.65 (m, CH<sub>2</sub>CH=CH<sub>2</sub> (both anomers overlapping), 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\alpha$ -anomer: 60.8 (C6), 98.2 (C1), 114.5 (CH<sub>2</sub>CH=CH<sub>2</sub>), 137.5 (CH<sub>2</sub>CH=CH<sub>2</sub>);  $\beta$ -anomer: 61.1 (C6), 102.4 (C1), 114.5 (CH<sub>2</sub>CH=CH<sub>2</sub>), 137.5 (CH<sub>2</sub>CH=CH<sub>2</sub>); Anal. Calc. for C<sub>11</sub>H<sub>20</sub>O<sub>6</sub>: C, 53.2; H, 8.10. Found: C, 53.3; H, 8.3%.

### Method 7

Preparation of *n*-pentenyl glycosides: Lewis acid mediated glycosylation of acetyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl- $\alpha$ -D-mannopyranoside with 4-penten-1-ol [58].



i) *n*-pentenol, SnCl<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, overnight; ii) NaOMe, MeOH, rt, 2h

*Notes and discussion.* Tin(IV) chloride can be used to catalyse the addition of *n*-pentenol to glycosyl acetates to provide *n*-pentenyl glycosides. This provides an alternative to Fischer glycosylation of reducing sugars or Koenigs–Knorr glycosylation of glycosyl halides. In this procedure, following glycosylation, the acetate groups of the product are removed without isolating the initial product to give an NPG with two free hydroxyl groups. Subsequent dibromination of the

pentenyl function would yield a 'latent' glycosyl acceptor, prior to glycosylation with an 'active' NPG.

*Materials.*

Acetyl 3,6-tri- <i>O</i> -acetyl-2,4-di- <i>O</i> -benzyl- $\alpha$ -D-mannopyranoside [59] (5.38 g, 11.4 mmol)	irritant
4-Penten-1-ol (5 cm <sup>3</sup> , 48 mmol)	assume toxic
Anhydrous dichloromethane (distilled from calcium hydride, 30 cm <sup>3</sup> )	risk of irreversible effects
Tin(IV) chloride	toxic, causes burns
Anhydrous methanol (distilled from calcium hydride, 20 cm <sup>3</sup> )	highly flammable, toxic, toxic: danger of very serious irreversible effects
Sodium	causes burns, reacts violently with water, liberating highly flammable gases
Sodium bicarbonate (saturated aqueous solution)	assume toxic
Dichloromethane (100 cm <sup>3</sup> )	risk of irreversible effects, toxic
Sodium sulfate	irritant
Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful

*Equipment.*

Round-bottomed flask (100 cm<sup>3</sup>) with rubber septum and magnetic stirrer bar  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) as inert gas  
 Syringe (5 cm<sup>3</sup>) and needle  
 Separatory funnel (250 cm<sup>3</sup>)  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Special care should be taken when handling sodium and it should be kept under oil or petrol as much as possible. It can be measured out by dropping small pieces (which have been dabbed dry on a paper towel) into a small beaker filled with petrol resting on the balance. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard.



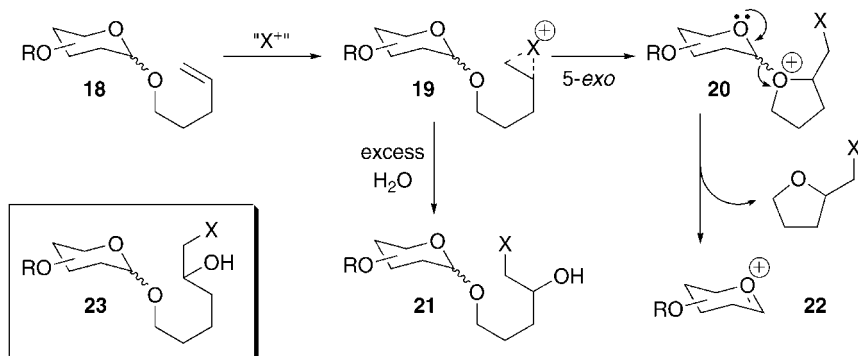
*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush the flask with argon. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add acetyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl- $\alpha$ -D-mannopyranoside (5.38 g, 11.4 mmol) by dissolving in anhydrous dichloromethane (30 cm<sup>3</sup>) under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Using a dry syringe add 4-penten-1-ol and then tin(IV) chloride. After stirring overnight the reaction will be complete, as evidenced by TLC analysis (petroleum ether/ethyl acetate 2:1). The reaction is worked-up by adding water (30 cm<sup>3</sup>) and then saturated aqueous sodium bicarbonate solution (50 cm<sup>3</sup>) to neutralise the mixture. The whole solution should now be transferred into a separatory funnel, dichloromethane (100 cm<sup>3</sup>) should be added and the lower organic layer should be collected and dried with sodium sulfate. Removal of the solvent *in vacuo* affords the crude product which is partially purified by passing through a short column of silica gel using petroleum ether/ethyl acetate 2:1, as the eluent. This intermediate product is dissolved in anhydrous methanol (20 cm<sup>3</sup>) and transferred to a round-bottomed flask with a magnetic stirrer bar and a rubber septum under argon. A small chip of sodium (0.026 g, 1.14 mmol) is added. After stirring for 2 h the reaction will be complete, as evidenced by TLC analysis (petroleum ether/ethyl acetate 1:1). Removal of the solvent *in vacuo* affords the crude reaction mixture. The desired product may be separated by column chromatography using silica gel and petroleum ether/ethyl acetate 1:1, as the eluent. This gives the desired product as an oil in 80% yield.  $[\alpha]_D^{22} = +28.5$  (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 1.60–1.70 (m, CH<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>, 2H), 2.06–2.20 (m, CH<sub>2</sub>CH=CH<sub>2</sub>, 2H), 3.34–3.41 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, 1H), 3.59–3.88 (m, 6H), 3.99–4.05 (dd, *J* = 3.5, 9.0 Hz, H5, 1H), 4.60–4.75 (m, PhCH<sub>2</sub>, 3H), 4.85 (s, H-1, 1H), 4.90–4.95 (m, PhCH<sub>2</sub>, 1H), 4.98–5.05 (m, CH<sub>2</sub>CH=CH<sub>2</sub>, 2H), 5.74–5.78 (m, CH<sub>2</sub>CH=CH<sub>2</sub>, 1H), 7.25–7.41 (m, ArH, 10H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) 97.1 (C-1), 115.1 (CH<sub>2</sub>CH=CH<sub>2</sub>), 137.8 (CH<sub>2</sub>CH=CH<sub>2</sub>).

### 5.2.2 Glycosylation chemistry of *n*-pentenyl glycosyl donors

Although the anomeric *n*-pentenyl group of an NPG is usefully inert under a wide variety of conditions, the application of halonium ions (specifically bromonium and iodonium ions) can bring about liberation of the anomeric substituent to produce a glycosyl cation (Scheme 5.1) which can be trapped by an aglycon alcohol. Originally, *N*-bromosuccinimide (NBS) was employed to activate NPGs. However, NBS alone (similarly *N*-iodosuccinimide (NIS) alone) promotes activation of NPGs only very slowly [52]. Thus, Fraser-Reid turned to the use of stronger activating systems, initially employing iodonium di-*sym*-collidine perchlorate [60, 61]. However IDCP, although certainly a stronger activator of NPGs than NBS, still struggles to find utility in very unreactive systems. Additionally, 'stalling' of

glycosylation reactions with IDCP is often observed, possibly due to liberation of free collidine [47]. The development of the strongly activating mixture of NIS and triflic acid meant that even unreactive NPGs could be activated almost instantaneously [62, 63]. However, triflic acid is troublesome to handle and the activator system of choice is, in fact, the similarly active NIS-triethylsilyltriflate (TES triflate) system [58, 64]. Both of these systems have a similar mode of action, in that the *N*-iodosuccinimide is activated towards release of the iodonium ion via coordination of a catalytic acidic species (i.e. proton or silyl triflate). In general practical terms, the glycosyl acceptor is usually mixed with NIS and TES triflate in dichloromethane at room temperature and the NPG donor is added. In many cases the coupling reaction is over before thin layer chromatography of the reaction mixture may be completed. Finally, it has also been shown that molecular iodine itself can activate NPGs to glycosylation, although at the time of writing this method has yet to be of proven synthetic utility [65]. The reaction of certain NPGs with bromine (alone) can be a route to glycosyl bromides (which can be used as glycosyl donors themselves under Koenigs–Knorr conditions) [66].

The activation mechanism of NPGs has been comprehensively studied, specifically for the case of the hydrolysis reaction (Scheme 5.7) [47]. Thus, after formation of the 4,5-cyclic halonium ion **19**, attack by the exocyclic oxygen atom (in a fast 5-*exo*-tet cyclisation process [67]) to give **20** is rapid, relative to the alternative possibility of direct trapping of **19** by the nucleophile to give **21**. Evidence for this includes the fact that the bromohydrin **21** is observed when the concentration of water is increased and this pathway becomes more significant. Additionally, if the *n*-hexenyl glycoside is employed instead, bromohydrin **23** is the sole product, which is consistent with the slower 6-*exo* cyclisation process in this case [68, 69]. Once cyclised intermediate **20** has been formed, elimination of the tetrahydrofuran component leads to the free glycosyl cation **22**. This species is subsequently trapped by the nucleophilic component of the reaction mixture (water in the case of

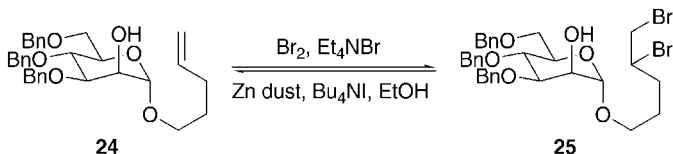


Scheme 5.7

hydrolysis, an alcohol during glycosylation, or a bromide anion during bromination). Further evidence for the formation of the glycosyl cation comes from the fact that the stereochemistry of the glycosylation reaction may be controlled to some extent by solvent effects. For example, use of diethyl ether as solvent often promotes  $\alpha$ -glycosidic products, whereas acetonitrile often gives rise to  $\beta$ -glycosides when it is the solvent [52, 70]. In a similar manner to trichloroacetimidate glycosylations (*vide supra*), the presence of a 2-*O*-acyl group on the glycosyl donor almost always leads to 1,2-*trans* glycosylated products, irrespective of solvent, through neighbouring group participation [71].

### 5.2.3 Reactivity of *n*-pentenyl glycosides in oligosaccharide synthesis i: latent and active *n*-pentenyl glycosides

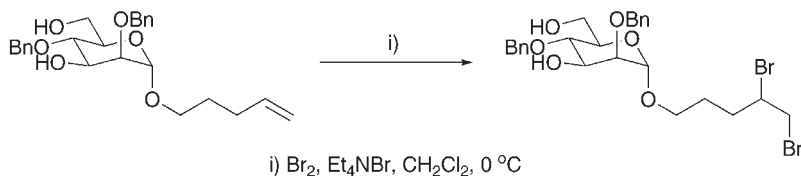
NPGs may be made inert to halonium ion activation via simple dibromination of the alkenyl function [72]. This may be useful if the NPG is actually to act as the glycosyl acceptor during a particular stage in the synthesis and inadvertent activation of the NPG would be undesirable. Thus, dibromination (e.g. **24**  $\rightarrow$  **25**) is brought about with the use of bromine and an additional bromide ion source (e.g. tetraethylammonium bromide, typically 0.5 equiv.) (Scheme 5.8) Use of bromine alone would generally result in loss of the pentenyl group and formation of the anomeric bromide (*vide supra*). It is of note that some very unreactive NPGs, for example *n*-pentenyl  $\alpha$ -mannosides, can give such dibromides even in the absence of additional bromide source, which is consistent with the mechanism for NPG activation discussed above. The dibromide is termed the 'latent' form of the NPG, whereas the NPG itself is the 'active' form [73] (Fraser-Reid calls this process 'sidetracking'). Not all NPGs undergo dibromination successfully using the standard conditions, and Fraser-Reid has also developed alternative conditions employing copper (II) bromide and lithium bromide which were used in the dibromination of an NPG containing a phthalimide residue [74]. The latent dibromide **25** may be converted to the active NPG **24** with zinc dust [72], samarium iodide or iodide ion [75]. Use of this latent/active strategy provides a method for the chemoselective assembly of oligosaccharides (*vide infra*).



Scheme 5.8

*Method 8*

Latent/active *n*-pentenyl glycosides: dibromination of (pent-4-enyl) 2,4-di-*O*-benzyl- $\alpha$ -D-mannopyranoside [47].



*Notes and discussion.* This procedure is used to convert an ‘active’ *n*-pentenyl glycoside into a ‘latent’ dibrominated glycoside. An additional bromide ion source is employed to aid trapping of the bromonium intermediate which forms on the alkenyl function of the pentenyl group when it reacts with molecular bromine. Such a dibromide is inert to the conditions which would otherwise activate an *n*-pentenyl glycoside during a NPG glycosylation reaction. Although stable under such glycosylation conditions, it is of note that dibromides may be affected by strongly basic conditions, such as sodium methoxide (often employed for deacetylation reactions, but can bring about elimination of HBr from a dibromide [58]).

*Materials.*

(Pent-4-enyl) 2,4-di- <i>O</i> -benzyl- $\alpha$ -D-mannopyranoside [58] (3.90 g, 9.11 mmol)	irritant
Tetraethyl ammonium bromide (1.47 g, 4.55 mmol)	irritant
Bromine (1.46 g, 9.11 mmol)	very toxic, causes severe burns
Anhydrous dichloromethane (distilled from calcium hydride, $20\text{ cm}^3$ )	risk of irreversible effects, toxic
Sodium thiosulfate (10% weight/volume aqueous solution)	irritant
Sodium sulfate	irritant
Ethyl acetate and petroleum ether (bp $40\text{--}60\text{ }^\circ\text{C}$ ) for chromatography	highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful

*Equipment.*

- Round-bottomed flask ( $100\text{ cm}^3$ ) with rubber septum and magnetic stirrer bar
- Magnetic stirrer
- Source of dry argon (or nitrogen) as inert gas
- Ice bath
- Syringe and needle

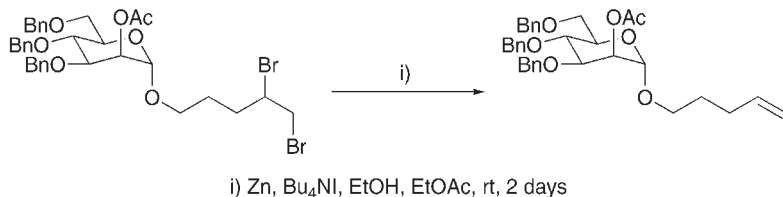
Separatory funnel (100 cm<sup>3</sup>)  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard.

*Procedure.* Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush the flask with argon. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add (pent-4-enyl) 2,4-di-*O*-benzyl- $\alpha$ -D-mannopyranoside (3.90 g, 9.11 mmol), dichloromethane (20 ml) and tetraethyl ammonium bromide (1.47 g, 4.55 mmol). Begin stirring until all of the pentenyl mannoside is dissolved, then lower the flask into the ice bath and let it cool to 0 °C. Add the bromine by syringe dropwise, and allow the solution to decolourise after each addition (it will go brown initially). When the brown colour persists, the reaction is complete. The reaction is quenched by the addition of 10% aqueous sodium thiosulfate solution (20 cm<sup>3</sup>). The whole solution should now be transferred into a separatory funnel, and the lower organic layer should be collected and dried with sodium sulfate. Removal of the solvent *in vacuo* affords the crude product which may be purified by column chromatography using silica gel and petroleum ether/ethyl acetate 6:1 to 2:1, as the eluent. This gives the desired compound as a colourless oil in 82% yield.  $[\alpha]_D^{22} = +21.4$  (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 1.60–1.89 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CHBrCH<sub>2</sub>Br, 3H), 2.20–2.32 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CHBrCH<sub>2</sub>Br, 1H), 3.36–3.44 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, 1H), 3.57–3.89 (m, 8H), 3.96–4.02 (dd, *J* = 3.0, 8.5 Hz, 1H), 4.10–4.20 (m, 1H), 4.58–4.75 (m, PhCH<sub>2</sub>, PhCH, 3H), 4.85 (s, H-1, 1H), 4.88–4.93 (m, PhCH, 1H), 7.23–7.43 (m, ArH, 10H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) 97.1 (C-1).

### Method 9

Debromination of (4,5-dibromopentanyl) 2-*O*-acetyl-3,4,6-tri-*O*-benzyl- $\alpha$ -D-mannopyranoside [75].



*Notes and discussion.* This procedure is the most commonly used method to convert a 'latent' dibromopentanyl glycoside into an 'active' *n*-pentenyl glycoside. Where

treatment with zinc in ethanol is ineffective or causes side reactions, samarium (II) iodide in tetrahydrofuran or sodium iodide in methyl ethyl ketone are useful alternatives [75].

*Materials.*

(4,5-Dibromopentanyl) 2- <i>O</i> -acetyl-3,4,6-tri- <i>O</i> -benzyl- $\alpha$ -D-mannopyranoside [58, 75] (1 g, 1.4 mmol)	irritant
Aqueous hydrochloric acid (10% weight/volume)	toxic, causes burns
Acetone	irritant, risk of serious damage to eyes
Zinc dust (0.45 g, 6.9 mmol)	contact with water liberates flammable gas, spontaneously flammable in air
Tetrabutyl ammonium iodide (0.51 g, 1.4 mmol)	assume toxic
Ethanol (20 cm <sup>3</sup> )	assume toxic
Ethyl acetate	highly flammable, irritant, may cause drowsiness/dizziness
Sodium thiosulfate (10% weight/volume aqueous solution)	irritant
Celite <sup>®</sup>	harmful, possible risk of irreversible effects
Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, may cause drowsiness/dizziness; highly flammable, harmful

*Equipment.*

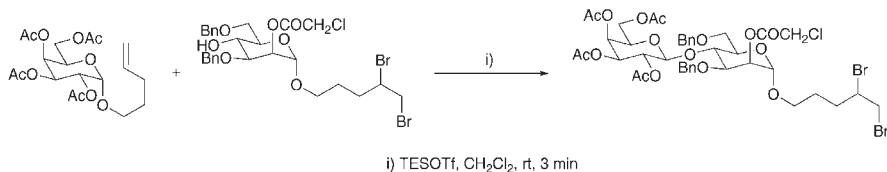
Round-bottomed flask (100 cm<sup>3</sup>) with rubber septum and magnetic stirrer bar  
 Source of dry argon (or nitrogen) as inert gas  
 High vacuum line  
 Sonicator  
 Syringe and needle  
 Facilities for carrying out NMR analysis  
 Separatory funnel (100 cm<sup>3</sup>)  
 Fritted glass funnel  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Glassware to be used under vacuum should be checked for star cracks before use. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard.

*Procedure.* Before the reaction is commenced, the oxide layer must be stripped from the zinc dust. This is achieved by stirring with aqueous hydrochloric acid (10% weight/volume) for 2 min, filtering, and then washing with water followed by acetone. Finally, the freshly activated zinc is dried under vacuum, and may be stored under an inert atmosphere before use [42]. Additionally, acquisition of  $^1\text{H}$  NMR data for the starting dibrominated material will prove useful for comparison during reaction monitoring later on. Equip a round-bottomed flask with a rubber septum and flush the flask with argon. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add (4,5-dibromopentanyl) 2-*O*-acetyl-3,4,6-tri-*O*-benzyl- $\alpha$ -D-mannopyranoside (1 g, 1.4 mmol), ethanol (20 cm<sup>3</sup>) and ethyl acetate to effect solubility, followed by zinc dust (0.45 g, 6.9 mmol) and tetrabutyl ammonium iodide (0.51 g, 1.4 mmol). The mixture is then sonicated to bring about reaction. After sonication for 2 days the reaction will be complete, as evidenced by  $^1\text{H}$  NMR analysis of a small portion of the mixture, removed via syringe and needle and concentrated *in vacuo*. Comparison with a spectrum of the starting material will show the disappearance of the peaks associated with the dibromopentanyl moiety and appearance of alkenyl peaks at 5.78 and 4.97 ppm. The reaction is worked-up by filtration through a bed of Celite<sup>®</sup> in a fritted glass funnel (about 2 cm high is sufficient; pre-pack the Celite<sup>®</sup> by running some ethyl acetate through it) and removal of the solvents *in vacuo*, followed by dilution with ethyl acetate (50 cm<sup>3</sup>), transferral to a separatory funnel and washing with 10% aqueous sodium thiosulfate solution (20 cm<sup>3</sup>). Removal of the solvent *in vacuo* affords the crude product which may be purified by column chromatography using silica gel and petroleum ether/ethyl acetate 4:1, as the eluent. This gives the desired compound as a colourless oil in 92% yield [58].  $[\alpha]_{\text{D}}^{22} = +23.2$  (c 1, CHCl<sub>3</sub>);  $^1\text{H}$  NMR (300 MHz, CDCl<sub>3</sub>) 1.65 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, 2H), 2.07 (m, CH<sub>2</sub>CH=CH<sub>2</sub>, 2H), 2.14 (s, OCH<sub>3</sub>, 3H), 3.40 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, 1H), 3.67 (m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, 1H), 3.68 (m, H-6, 1H), 3.78 (m, H-6, 1H), 3.79 (m, H-5, 1H), 3.86 (t,  $J = 9.5$  Hz, H-4, 1H), 3.97 (dd,  $J = 9.5, 3.5$  Hz, H-3, 1H), 4.40–4.87 (m, PhCH<sub>2</sub>, 6H), 4.82 (s, H-1, 1H), 4.97 (m, CH<sub>2</sub>CH=CH<sub>2</sub>, 2H), 5.34 (m, H-2, 1H), 5.78 (m, CH<sub>2</sub>CH=CH<sub>2</sub>, 1H), 7.15–7.39 (m, ArH, 15H);  $^{13}\text{C}$  NMR (75 MHz, CDCl<sub>3</sub>) 21.1 (OCH<sub>3</sub>), 28.5 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 30.2 (CH<sub>2</sub>CH=CH<sub>2</sub>), 67.2, 68.9, 71.3, 71.8, 73.4, 74.4, 75.2, 78.2 (C-2, C-3, C-4, C-5, C-6, OCH<sub>2</sub>CH<sub>2</sub>, 3  $\times$  PhCH<sub>2</sub>), 97.7 (C-1), 115.0 (CH<sub>2</sub>CH=CH<sub>2</sub>), 127.6, 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.3, 128.4 (Ar{C-2, C-3, C-4, C-5, C-6}), 137.9 (CH<sub>2</sub>CH=CH<sub>2</sub>), 137.9, 138.2, 138.3 (Ar{C-1}), 170.5 (COCH<sub>3</sub>); MS  $m/z$  (CI) 578 (M + NH<sub>4</sub>).

*Method 10*

Glycosylation reactions of *n*-pentenyl glycosides: glycosylation of (4,5-dibromopentanyl)-2-*O*-chloroacetyl-3,6-di-*O*-benzyl- $\alpha$ -D-mannopyranoside with pent-4-enyl 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranoside [76].



*Notes and discussion.* This procedure is one of many examples in the literature of a high yielding glycosylation of a latent NPG (i.e. dibrominated) with an active NPG (in this case a disarmed peracetylated galactoside). The standard activating conditions are used (*N*-iodosuccinimide and triethylsilyl triflate) and in this example the stereocontrolling element is the participating acetate group on *O*-2 of the donor, which facilitates production of the 1,2-*trans* product. It is of note that in this case the corresponding Koenigs–Knorr procedure with acetobromogalactose was ‘unsatisfactory’.

*Materials.*

(4,5-Dibromopentanyl)-2- <i>O</i> -chloroacetyl-3,6-di- <i>O</i> -benzyl- $\alpha$ -D-mannopyranoside [76] (0.86 g, 1.30 mmol)	irritant
Pent-4-enyl 2,3,4,6-tetra- <i>O</i> -acetyl- $\beta$ -D-galactopyranoside [77] (0.704 g, 1.69 mmol)	irritant
<i>N</i> -Iodosuccinimide (0.38 g, 1.69 mmol)	harmful, irritant
Triethylsilyl trifluoromethanesulfonate (TES triflate) (80 $\mu$ l, 0.39 mmol)	causes burns, reacts violently with water
Anhydrous dichloromethane (17 cm <sup>3</sup> , distilled from calcium hydride)	risk of irreversible effects
Sodium thiosulfate (10% weight/volume aqueous solution)	irritant
Sodium hydrogen carbonate (saturated aqueous solution) dichloromethane	risk of irreversible effects
1,4-Dioxane	may cause cancer, may cause heritable genetic damage, may form explosive peroxides, harmful, irritant



Carbon tetrachloride	may cause cancer, may cause heritable genetic damage, toxic, irritant
Ethyl acetate and petroleum ether (bp 40–60 °C) for chromatography	highly flammable, irritant, may cause drowsiness/ dizziness; highly flammable; harmful

*Equipment.*

Bunsen burner  
Round-bottomed flask (50 cm<sup>3</sup>) with a rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Syringe (5 cm<sup>3</sup>) and needle  
Microsyringe (100 µl) and needle  
Separatory funnel  
Rotary evaporator  
Column chromatography equipment

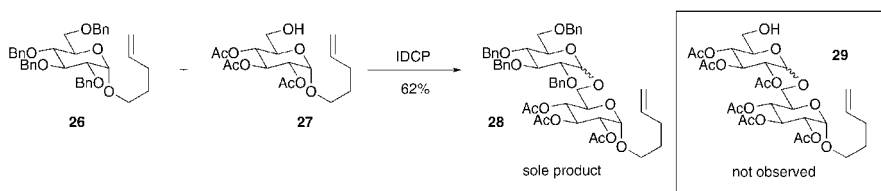
*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* *N*-Iodosuccinimide should be recrystallised in advance from dioxane-carbon tetrachloride. Equip a round-bottomed flask with a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add (4,5-dibromopentanyl)-2-*O*-chloroacetyl-3,6-di-*O*-benzyl- $\alpha$ -D-mannopyranoside (0.86 g, 1.30 mmol) to the flask by dissolving it in anhydrous dichloromethane (13 cm<sup>3</sup>) under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum). Quickly unstopper the flask to add *N*-iodosuccinimide (0.38 g, 1.69 mmol), re-stopper and flush with argon. Use a dry microsyringe to add TES triflate (80 µl, 0.39 mmol) which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. Dissolve pent-4-enyl 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranoside (0.704 g, 1.69 mmol) in anhydrous dichloromethane (4 cm<sup>3</sup>) under argon and add it dropwise to the reaction vessel via cannula under inert gas pressure at room temperature. After stirring for

3 min the reaction mixture will become a pale pink homogeneous solution and the reaction will be complete, as evidenced by TLC analysis (petroleum ether/ethyl acetate 3:1). The reaction is quenched by the addition of 10% aqueous sodium thiosulfate solution (20 cm<sup>3</sup>). The whole solution should now be transferred into a separatory funnel, and the lower organic layer should be collected, washed with saturated sodium bicarbonate solution, recollected and dried with sodium sulfate. Removal of the solvent *in vacuo* affords the crude product which may be purified by column chromatography using silica gel and petroleum ether/ethyl acetate 3:1 as the eluent. This gives the compound as an oil in 87% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 1.75–2.02 (m, 3H), 2.02 (s, CH<sub>3</sub>CO, 3H), 2.06 (s, 2 × CH<sub>3</sub>CO, 6H), 2.21 (s, CH<sub>3</sub>CO, 3H), 2.25–2.40 (m, 1H), 3.54–4.28 (m, 15H), 4.57–4.90 (m, H-1', 2 × PhCH<sub>2</sub>, 5H), 4.91–4.95 (m, H-1, H-3', 2H), 5.21 (dd, H-2', 1H), 5.36 (d, H-4', 1H), 5.43 (t, H-2, 1H), 7.37–7.46 (m, ArH, 10H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) 20.5, 20.6, 20.6, 20.8, 26.6 (CH<sub>3</sub>CO), 32.7, 35.9 (CH<sub>2</sub>Br), 40.7 (CH<sub>2</sub>Cl), 52.2 (CHBrCH<sub>2</sub>Br), 66.7 (C-4'), 67.1, 67.9 (C-6, C-6'), 69.5 (C-2'), 70.7 (C-2), 70.7, 70.9 (C-3'), 71.1, 71.8 (PhCH<sub>2</sub>), 73.6 (PhCH<sub>2</sub>), 74.2, 75.8, 97.2 (C-1), 100.5 (C-1'), 127.1–128.5 (Ar{C-2, C-3, C-4, C-5, C-6}), 138.0, 138.0 (Ar{C-1}), 166.6, 169.3, 170.0, 170.0, 170.1 (5 × C=O); Anal. Calc. for C<sub>41</sub>H<sub>51</sub>O<sub>16</sub>Br<sub>2</sub>Cl: C, 49.49; H, 5.17. Found: C, 49.57; H, 5.24%.

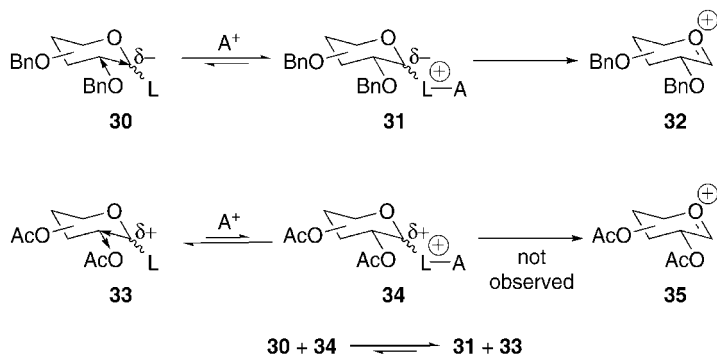
### 5.2.4 Reactivity of *n*-pentenyl glycosides in oligosaccharide synthesis ii: armed and disarmed *n*-pentenyl glycosides

More subtle than the latent/active approach for chemoselective glycosylation of NPGs is a second method which relies on the differential reactivity towards halonium ion sources that is a feature of differentially protected NPGs. In the most basic application of this method, NPGs with ether-based protecting groups react with promoting agents much more rapidly than those with ester-based protecting groups. Thus, in a typical reaction (Scheme 5.9) [61], only the benzylated NPG **26** is activated whilst the *n*-pentenyl group on the acetylated NPG **27** remains intact and a single product **28** is obtained. No cross-coupling (to give **29**) or trisaccharide is observed which are both theoretically possible, given the starting materials. This effect, which was also investigated for 2-deoxy-2-bromo versus 2-deoxy NPGs [61], was originally explained by Fraser-Reid through examination of the inductive effect



Scheme 5.9

of only the C-2 substituent (Scheme 5.10) [72]. (Since these initial disclosures and postulations, further work has shown that this explanation is only part of the picture, *vide infra*).

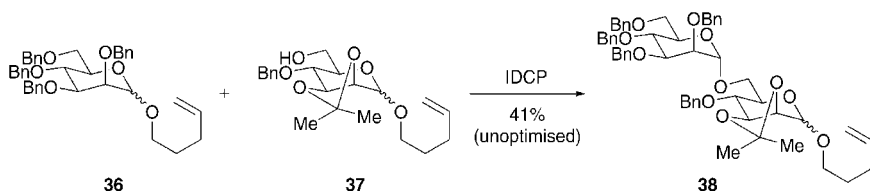


Scheme 5.10

In this initial explanation, the NPGs (**30** and **33**, L = *n*-pentyloxy) compete for the electrophilic activator in solution. The rate limiting step in the formation of the glycosyl cation (**32** or **35**) therefore involves the build-up of positive charge in the transition state, concentrated around the anomeric position. Intermediate **34**, with an electron withdrawing acyl group at C-2 and a partial positive charge at the anomeric position is therefore destabilised relative to intermediate **31**, where the electron donating group at C-2 ensures a partial negative charge at the anomeric position which stabilises the positively charged intermediate. Thus **30** reacts faster with the electrophilic activator ( $A^+$ ), to form glycosyl cation **32** which may be trapped by the acceptor sugar. Since no cross coupling product is seen, which suggests that **34** does not form appreciable amounts of the glycosyl cation, it is also likely that an equilibrium exists between **31** and **34**, in which **34** essentially transfers the electrophilic group to reactive NPG **30** (to give **31**) as a consequence of their relative stabilities. Fraser-Reid termed the more reactive NPG the ‘armed’ glycoside, whilst the less reactive NPG was called the ‘disarmed’ glycoside. These terms were used, rather than ‘activated’ and ‘deactivated’ principally to avoid confusion with other conventions, but also because even disarmed NPGs can be activated by suitably strong activating conditions [78]. It is of note, however, that the utility of armed/disarmed NPGs in glycosylation reactions is not dependent on the promoter which is used, as this merely effects the speed with which the reaction is complete: the armed NPG will react preferentially [63].

Fraser-Reid’s early work with armed/disarmed NPGs quantified effects that were already known at the time, but had not been effectively exploited for glycosylation chemistry [79–81]. Subsequent to the work with NPGs, similar effects were recorded for other donors. The concept has been extended well

beyond the ester versus ether effect, such that a full treatment of the factors and possibilities involved is given elsewhere (Chapter 7). In particular, the effect of torsional interactions on the reactivity of the donor sugar has been examined both experimentally and with the help of molecular modelling [82, 83]. Essentially, cyclic protecting groups can disarm the donor by introducing torsional strain into the glycosyl cation and thus making its formation energetically less favourable. Scheme 5.11 shows the application of this effect in a synthesis of a disaccharide with NPGs [82]. In practice, the upshot of these studies is that chemoselective glycosylation sequences using armed and disarmed donors are possible with careful planning (for both *n*-pentenyl glycosides and other glycosyl donors—for good examples of such ‘reactivity tuning’, see Refs. 84, 85).



Scheme 5.11

### 5.2.5 Glycosylation reactions with *n*-pentenyl glycosides: summary

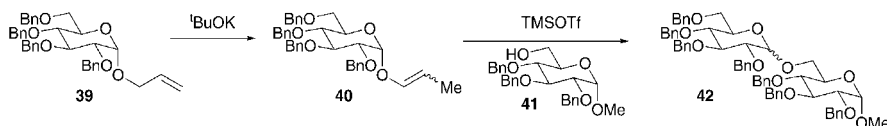
It is interesting to note that Fraser-Reid's *n*-pentenyl glycoside methodology has provided the basis for other glycosylation techniques, for example *n*-pentenyl ester-based glycosides [86, 87], *N*-allyl carbamate-based glycosides [88] and *S*-pent-4-enyl thioglycosides [89]. Additionally, a pendant *n*-pentenyl group at the anomeric position has been shown to be a useful precursor for the attachment of glycosides to proteins [90], fluorescence labels [91], carbosilane dendrimers [92] and as a precursor to other spacer functionalities [93]. However, the true usefulness of the NPG methodology still lies in its power as a glycosylation system, the prime advantages of which are the fact that the *n*-pentenyl group is inert to many synthetic manipulations but may be activated upon demand; when required, the active NPG may be made latent via dibromination; and the reactivity may be ‘tuned’ by careful selection of the protecting groups employed.

## 5.3 USE OF VINYL GLYCOSIDES AS GLYCOSYL DONORS

The application of vinyl glycosides as glycosyl donors has, overall, been less successful than that of trichloroacetimidates or *n*-pentenyl glycosides. However, several different approaches have been taken by a number of different research groups and the work of Boons and co-workers in particular has led to some useful methodology with general applicability in oligosaccharide assembly.

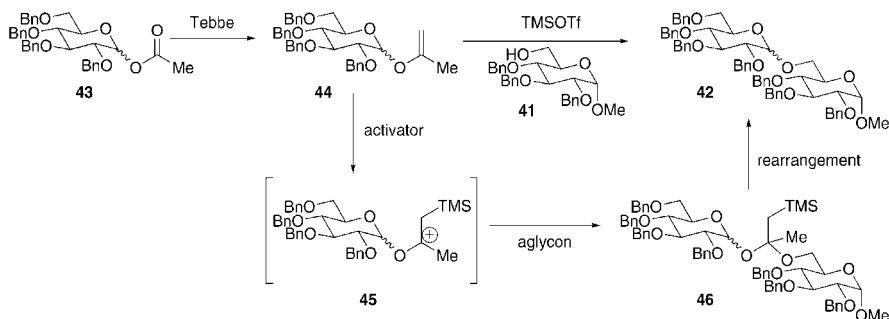
### 5.3.1 Isopropenyl glycosides as glycosyl donors

The use of vinyl glycoside donors was first reported in 1991 independently by both Sinaÿ and Schmidt [36]. Sinaÿ's work, continued in 1992 [94], initially focused on the use of a prop-1-enyl glycoside **40** (obtained via isomerization of an allyl glycoside **39**), which was activated towards glycosylation with TMS triflate (Scheme 5.12). Attention shifted to the use of isopropenyl glycosides when it was felt that such donors would be more active due to the additional stability of the intermediate cationic species **45** brought about by the position of the electron donating methyl group relative to the developing positive charge (Scheme 5.13).

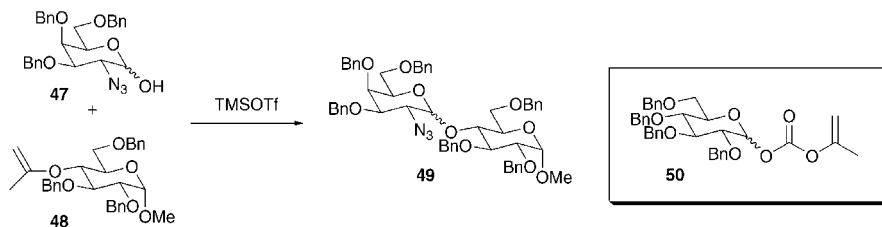


Scheme 5.12

The isopropenyl glycosides were prepared by subjecting the corresponding acetate to Tebbe methylenation (e.g. **43** → **44**) which, although high yielding, can be potentially troublesome to carry out. The activating agent of choice was again TMS triflate which, in general, produced reasonable yields of around 70% for a range of systems. Sinaÿ's work suggested that the glycosylation pathway for these vinyl glycosides proceeded via a mixed acetal intermediate **46**. With this in mind, the so-called 'reverse approach' was possible, in which vinyl ether **48** was condensed with hemi-acetal **47**, again using TMS triflate (Scheme 5.14). This was required as the azido function of **47** was unstable to treatment with the Tebbe reagent. Sinaÿ also included results that illustrated the use of the related isopropenyl carbonate **50** (Scheme 5.14), which could be prepared without recourse to Tebbe methylenation via treatment of the corresponding carbohydrate hemiacetal with isopropenyl chloroformate. Glycosylation of such species with both primary and secondary carbohydrate alcohol acceptors was relatively high yielding (around 80%). All three variations on this theme showed mild, solvent-dependent stereoselectivity during the glycosylation step.

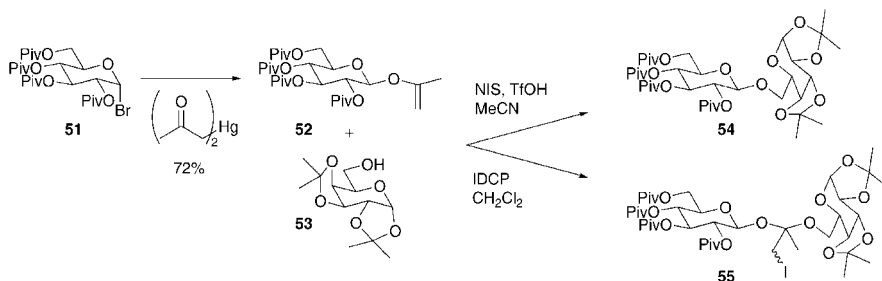


Scheme 5.13



Scheme 5.14

Chenault and co workers have extended Sinaÿ's work with isopropenyl glycosides, confirming the proposed mechanistic pathway [95]. Rather than utilise Tebbe methylenation, the reaction of bis(acetonyl)mercury with glycosyl halides was instead used to prepare the vinyl glycosides (e.g. **51** → **52**, Scheme 5.15). This method permits the inclusion of ester groups in the donor molecule, which the Tebbe reagent, and the related Petasis reagent, do not. The vinyl glycosides so prepared are stable, readily purified on silica gel and can be stored at room temperature for extended periods of time. Successful glycosylation of these isopropenyl glycosides could be brought about by the action of NIS/triflic acid, TMS triflate, triflic anhydride and dimethyl(methylthio)sulfonium triflate (DMTST). However conditions which favour formation of the glycosyl cation (polar solvent, e.g. acetonitrile, and a strong electrophilic promoter) are essential, otherwise side products predominate, specifically addition of electrophile and acceptor alcohol across the vinyl ether double bond (e.g. **55**, Scheme 5.15)—corresponding to intermediate **46** in Scheme 5.13.

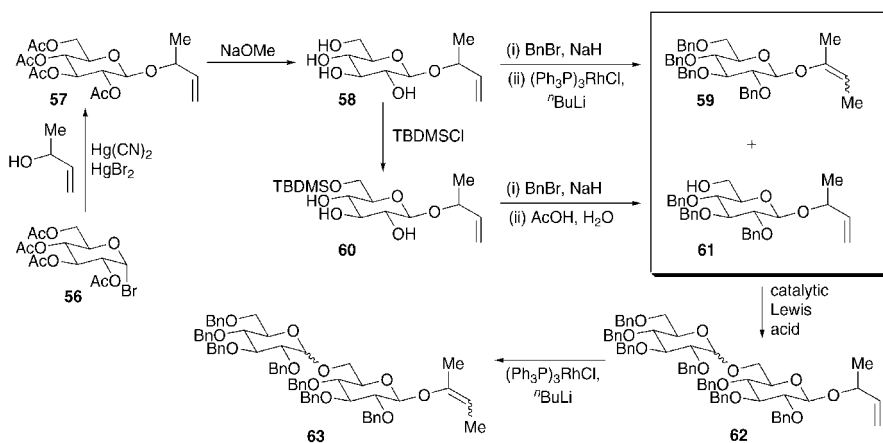


Scheme 5.15

Although the methodology introduced by Sinaÿ and advanced by Chenault does essentially work, glycoside synthesis using such vinyl ethers has no definite advantage over methods such as the trichloroacetimidate or *n*-pentenyl approaches detailed above and suffers from relatively non-trivial procedures required in the preparation of the donor species. However, a useful application of vinyl glycosides in oligosaccharide synthesis has subsequently been developed and applied successfully by Boons.

### 5.3.2 A latent/active glycosylation strategy using vinyl glycosides

In a modification of Sinaÿ's approach, Boons reported the glycosylation chemistry of 2-isobutenyl glycosides, which can be obtained via isomerization of the corresponding 3-buten-2-yl glycosides [96, 97] (e.g. **58**  $\rightarrow$  **59**, Scheme 5.16). These allyl glycosides can themselves be obtained through Koenigs–Knorr glycosylation of glycosyl bromides with 3-buten-2-ol. Standard manipulation of protecting groups after addition of the butenyl function can provide glycosides with a diverse range of protecting group patterns suitable for oligosaccharide synthesis. Boons has also shown that such butenyl glycosides may be obtained enzymatically from the unprotected carbohydrate [98]. Although Boons' use of racemic 3-buten-2-ol complicates the interpretation of NMR spectra of the glycosides involved, this does not affect the chemistry in any way. It is possible to obtain multigram quantities of optically pure 3-buten-2-ol if required for interpretive purposes [99]. Boons' system provides an excellent example of the 'latent/active' approach to chemoselective glycosylation (Scheme 5.16).



Scheme 5.16

Thus 3-buten-2-yl glycoside **61** is inert towards activation, whereas the isomerized 2-isobutenyl glycoside **59** may be activated using catalytic amounts of TMS triflate, boron trifluoride etherate or NIS/triflic acid. Note that 2-isobutenyl glycosides are more reactive towards electrophilic activation than their isopropenyl counterparts due to the presence of an extra electron donating methyl group on the vinyl ether. Once the first glycosylation has taken place, the latent anomeric group of the product (e.g. **62**) can also be isomerized in an efficient fashion to provide the active form (e.g. **63**) which is then ready for glycosylation with a second latent acceptor.

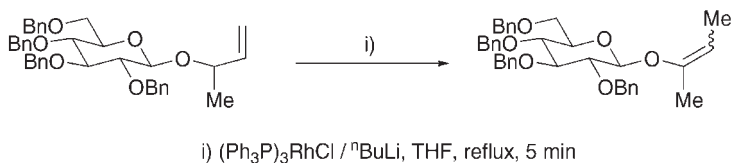
One of the key features of Boons' approach is the efficiency of the isomerization reaction. Early attempts to use Wilkinson's catalyst with a hindered base (DABCO)

were successful but often led to side reactions. Thus a modified procedure was adopted in which Wilkinson's catalyst is dissolved in freshly distilled tetrahydrofuran, degassed and treated with *n*-butyllithium. This forms a deep red solution of the active catalytic species, which appears to be the rhodium hydride  $(\text{Ph}_3\text{P})_3\text{RhH}$  [97]. This is able to isomerize allyl functions to vinyl ethers, often almost instantaneously, with essentially no side reactions. Practically very easy to perform, it is even possible to carry out the glycosylation of the freshly isomerized donor in the same pot, assuming that the catalyst is exposed to oxygen first. Of note also is the fact that base labile functions are stable under these conditions.

The stereochemical outcome of glycosylation reactions carried out using these conditions follows similar rules to those for *n*-pentenyl glycosides and trichloroacetimidates. A 2-*O*-acyl protecting group on the donor will usually force a 1,2-*trans* glycosylation; otherwise solvent effects play a part, acetonitrile promoting  $\beta$ -products and diethyl ether promoting  $\alpha$ -products [97]. Boons has also shown that a dioxane/toluene solvent mixture is often superior to diethyl ether for the promotion of  $\alpha$ -product formation [100, 101].

#### Method 11

Latent/active strategy using vinyl glycosides: isomerization of 3-buten-2-yl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranoside [97].



**Notes and discussion.** This is the procedure optimised by Boons and Isles for converting a latent allyl glycoside into an active vinyl glycoside. It is superior to previously reported and applied techniques for isomerizing allyl functions as the reaction is usually over in minutes, essentially no hydrolysis of the product enol ether occurs and the product mixture may even be used crude for the glycosylation step that follows. If purification is required, then this is easily achieved through silica gel chromatography of the crude product. The catalytic species is assumed to be a rhodium hydride formed through the reaction of Wilkinson's catalyst with the butyl anion (since Wilkinson's catalyst and *n*-butyl lithium are unable to isomerize allyl functions when used on their own).

#### Materials.

3-Buten-2-yl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranoside [97] (0.20 g, 0.33 mmol)

irritant

*n*-Butyl lithium (1.6 M solution in hexanes, 28  $\mu\text{l}$ , 45  $\mu\text{mol}$ )

highly flammable, contact with water liberates



	extremely flammable gases, spontaneously flammable in air, causes burns, harmful
Wilkinson's catalyst (0.03 g, 33 $\mu\text{mol}$ )	harmful
Anhydrous tetrahydrofuran (THF) (2.5 $\text{cm}^3$ , distilled from sodium/benzophenone)	highly flammable, may form explosive peroxides, irritant
Dichloromethane (10 ml)	risk of irreversible effects, toxic
Petroleum ether (bp 40–60 $^{\circ}\text{C}$ )	highly flammable, harmful
Acetone and dichloromethane for chromatography	highly flammable, irritant, may cause drowsiness or dizziness; possible risk of irreversible effects

### *Equipment.*

Bunsen burner

Two-necked round-bottomed flask (25  $\text{cm}^3$ ) with a rubber septum in one neck and Teflon tap in the second neck, plus magnetic stirrer bar

Two-necked round-bottomed flask (25  $\text{cm}^3$ ) with a rubber septum in one neck and water condenser with rubber septum in the second neck, plus magnetic stirrer bar

Magnetic hotplate stirrer with silicone oil heating bath and temperature probe

Source of dry argon (or nitrogen) as inert gas

High vacuum line

Short cannula (approx. 10 cm long)

Syringe (5  $\text{cm}^3$ ) and needle

Microsyringe (50  $\mu\text{l}$ ) and needle

Round-bottomed flask (50  $\text{cm}^3$ )

Rotary evaporator

Column chromatography equipment

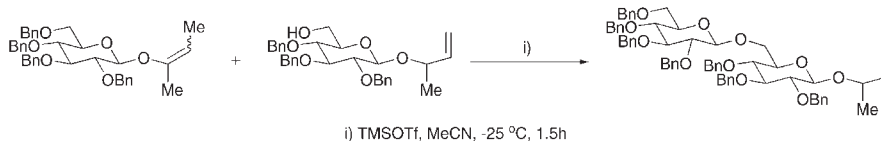
*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Glassware to be used under vacuum should be checked for star cracks before use. Additionally, once the glassware has been assembled and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

*Procedure.* Before the reaction is commenced, the *n*-butyl lithium solution should be standardised to known concentration by titrating against an indicator such as 2,2,2'-trimethylpropionanilide [102]. Before using *n*-butyl lithium, the bottle must be clamped still and a beaker of petroleum ether made ready to dilute the excess alkyl lithium remaining in the syringe. Equip a two-necked flask with a water condenser (topped with a rubber septum), magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add the 3-buten-2-yl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranoside (0.20 g, 0.33 mmol) in THF (1 cm<sup>3</sup>) and then lower the flask into the heating bath, stir and heat to reflux (set the heating temperature to approx. 70 °C). Whilst the temperature is increasing to 70 °C, take a second two-necked flask equipped with a Teflon tap, a magnetic stirrer bar and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. Add the Wilkinson's catalyst (0.03 g, 33  $\mu$ mol) to this second flask and then THF (1.5 cm<sup>3</sup>) to give a light red solution. Attach a balloon of argon to the Teflon tap and close the tap. Using a needle attached to a vacuum outlet and piercing the septum, expose the solution to vacuum until it begins to bump. Immediately close-off the vacuum and open the Teflon tap to permit argon to enter the flask. Repeat this until the bumping is very slow to start—the solution is now degassed. Leaving the solution under argon, add the *n*-butyl lithium (1.6 M solution in hexanes, 28  $\mu$ l, 45  $\mu$ mol) via dry microsyringe, taking the precautions noted above. (Once addition of alkyl lithium is complete, immediately flush the syringe with petrol from the beaker to dilute remaining reagent and prevent the formation of lithium residues; for a larger scale reaction, this dilute solution could be quenched with isopropanol.) During addition of *n*-butyl lithium to the Wilkinson's catalyst solution, the solution will turn a darker shade of red which is subtle but noticeable. Allow this solution to stir under argon for 5 min before adding to the refluxing solution of butenyl glycoside via cannula under inert gas pressure (an exit needle or bubbler will be required in the condenser septum). After 5 min the reaction will be complete, as evidenced by TLC analysis (dichloromethane). Remove the flask from the heating bath and allow to cool to room temperature before the addition of dichloromethane (10 cm<sup>3</sup>). Transfer the solution to a round-bottomed flask and concentrate *in vacuo* to afford a crude red oil. The residue may be purified by column chromatography using silica gel and dichloromethane to give the desired compound as a mixture of *cis* and *trans* isomers, as a colourless oil in 91% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.59 (2  $\times$  q, *J* = 7.0, 1.1 Hz, CH<sub>3</sub> (*cis*), 1.5H), 1.62 (2  $\times$  q, *J* = 7.0, 1.5 Hz, CH<sub>3</sub> (*trans*), 1.5H), 1.86 (m, CH<sub>3</sub> (*cis*), 1.5H), 1.92 (m, CH<sub>3</sub> (*trans*), 1.5H), 3.43 (m, H-5, 1H), 3.76–3.58 (m, H-2, H-3, H-4, 2  $\times$  H-6, 5H), 4.75 (d, *J* = 8.0 Hz, H-1, 1H), 4.86 (m, 4  $\times$  PhCH<sub>2</sub>, 8H), 4.90 (m, C=CH, 1H), 7.40–7.23 (m, ArH, 20H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) 10.5, 12.0 (CHCH<sub>3</sub>), 15.7, 18.7 (CH<sub>3</sub>), 69.6 (C-6), 73.5, 74.9, 75.0 (PhCH<sub>2</sub>), 77.9, 80.9, 82.0, 84.9 (C-2, C-3, C-4, C-5), 100.2, 100.5 (C-1), 100.5, 105.1 (CHCH<sub>3</sub>), 128.2–127.1 (Ar{C-2, C-3, C-4, C-5, C-6}), 138.7–138.2 (Ar{C-1}), 149.4 (OC(CH<sub>3</sub>)=C); MS *m/z* 617 (M + Na, 100%), 593 (MH, 7), 523 (M – [*O*-2-buten-

2-yl], 12), 415 (M – H-[O-Bn]-[O-2-buten-2-yl], 19), 271 (M – H-3[O-Bn], 70), 253 (M – H-2[O-Bn]-[O-2-buten-2-yl], 17);  $C_{38}H_{42}O_6Na$  requires 617.2879, found 617.2895.

### Method 12

Latent/active strategy using vinyl glycosides: glycosylation of ‘active’ donor 2-buten-2-yl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranose with ‘latent’ acceptor (*R/S*)-3-buten-2-yl 2,3,4-tri-*O*-benzyl- $\beta$ -D-glucopyranoside [97].



**Notes and discussion.** This is the procedure developed by Boons and Isles for the glycosylation of an ‘active’ vinyl glycoside with a ‘latent’ butenyl acceptor. Isomerization of the latent allylic anomeric substituent of the product disaccharide may also give an active vinyl glycoside for iterative glycosylation. It is of note that the anomeric stereospecificity of the glycosylation may be controlled to a certain degree via a change of solvent. Thus, the detailed procedure, which invokes acetonitrile as solvent, provides a predominance of  $\beta$ -product (1:20,  $\alpha/\beta$ ). However, when the reaction is performed in dichloromethane, almost equal amounts of each anomer are formed (4:3,  $\alpha/\beta$ ). Finally, when the reaction is performed with a 1:1 mixture of ether and dichloromethane as the solvent (and NIS/triflic acid as promoter), the  $\alpha$ -anomer of the product is formed in greater amount (4:1,  $\alpha/\beta$ ). It is also interesting that this glycosylation may be performed using the crude product from the isomerization (rather than ‘pure’ vinyl glycoside) simply by exposing the completed isomerization reaction mixture to oxygen for 30 min before addition to the acceptor alcohol (in the solvent of choice).

### Materials.

2-Buten-2-yl 2,3,4,6-tetra- <i>O</i> -benzyl- $\beta$ -D-glucopyranose [97] (0.285 g, 0.48 mmol)	irritant
( <i>R/S</i> )-3-Buten-2-yl 2,3,4-tri- <i>O</i> -benzyl- $\beta$ -D-glucopyranoside [97] (0.200 g, 0.40 mmol)	irritant
Trimethylsilyl trifluoromethanesulfonate (TMS triflate) (20 $\mu$ l, 0.10 mmol)	causes burns, flammable
Powdered molecular sieves, 4 Å	irritant
Anhydrous acetonitrile (5 cm <sup>3</sup> , distilled from calcium hydride)	highly flammable, toxic

Triethylamine (50 $\mu$ l)	highly flammable, harmful, causes severe burns
Dichloromethane (25 $\text{cm}^3$ )	risk of irreversible effects, toxic
Celite <sup>®</sup>	harmful, possible risk of irreversible effects
Acetone, dichloromethane and methanol for chromatography	highly flammable, irritant, may cause drowsiness or dizziness; possible risk of irreversible effects; highly flammable, toxic, toxic: danger of very serious irreversible effects

*Equipment.*

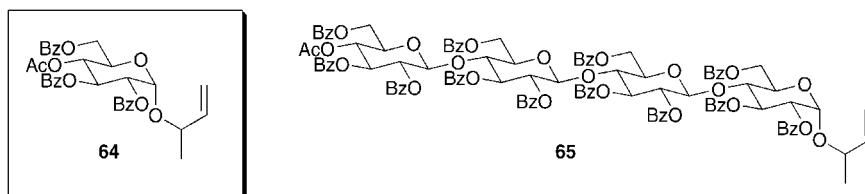
Bunsen burner  
Round-bottomed flask (25  $\text{cm}^3$ ) with a rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon (or nitrogen) as inert gas  
Cooling bath for 25 °C [42]  
Syringe (5  $\text{cm}^3$ ) and needle  
Microsyringe (50  $\mu$ l) and needle  
Fritted glass funnel  
Round-bottomed flask (50  $\text{cm}^3$ )  
Rotary evaporator  
Sephadex chromatography equipment

*Special precautions.* All glassware must be dried in an oven before use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. 4 Å molecular sieves should be freshly activated before use by heating in a furnace for 3 h at 330 °C [103]. They may be measured out of the furnace and into a warm flask as swiftly as possible by collecting in and dispensing from the wide end of a Pasteur pipette (by measuring the depth of the required sample cold, an approximately accurate mass may be dispensed). Additionally, once the glassware has been assembled (with sieves incorporated) and placed under inert atmosphere, but before the addition of any solvents or reagents, it should be flame dried using the Bunsen burner in tandem with a flow of inert gas. Care should be taken not to use a naked flame in the proximity of flammable solvents or oils. A 25 °C cooling bath may be prepared with the use of a solid carbon dioxide/carbon tetrachloride mixture in a dewar, although a safer alternative is a carefully monitored mixture of solid carbon dioxide and isopropanol. Safety glasses should be worn during this experiment and all manipulations should be performed in a fume cupboard whilst wearing protective gloves.

**Procedure.** Equip a round-bottomed flask with a magnetic stirrer bar, 4 Å molecular sieves (ca. 0.45 g) and a rubber septum and flush this with argon, then flame dry it and allow to cool, maintaining the inert atmosphere. A balloon of argon can be used to maintain the inert atmosphere during the reaction. Add 2-buten-2-yl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranose (0.285 g, 0.48 mmol) and (*R/S*)-3-buten-2-yl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (0.200 g, 0.40 mmol) to the flask by dissolving each in anhydrous acetonitrile under argon and adding to the reaction vessel via cannula under inert gas pressure (an exit needle or bubbler will be required in the receiving flask's septum)—use a total of 5 cm<sup>3</sup> of acetonitrile. Stir this solution for 30 min and then cool it to 25 °C by immersing in a cooling bath. Use a dry microsyringe to add TMS triflate (20 μl, 0.10 mmol) which must be handled carefully to prevent its (rapid) reaction with atmospheric moisture. After 90 min the reaction will be complete, as evidenced by TLC analysis (acetone/dichloromethane 3:97). The reaction is worked-up by the syringe addition of triethylamine (50 μl) to neutralise the mixture, dilution with dichloromethane (25 cm<sup>3</sup>) and filtration through a bed of Celite® in a fritted glass funnel (about 2 cm high is sufficient; pre-pack the Celite® by running some dichloromethane through it). Removal of the solvent *in vacuo* affords the crude product as a solid residue. The product may be isolated in pure form by passing down a column of Sephadex (100 g, methanol/dichloromethane 1:1 as eluent) which gives the product as a 1:20 mixture of α- and β-anomers in 78% yield. α-Anomer: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.30 (t, *J* = 4.0 Hz, CHCH<sub>3</sub>, 3H), 3.73–3.32 (m, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', 2 × H-6, 2 × H-6', 12H), 4.26 (t, *J* = 4.0 Hz, H-1', 1H), 4.30 (m, CHCH<sub>3</sub>, 1H), 4.49 (d, *J* = 8.0 Hz, H-1, 1H), 4.98–4.53 (m, 7 × PhCH<sub>2</sub>, 14H), 5.21–5.00 (m, CH=CH<sub>2</sub>, 2H), 5.99–5.72 (m, CH=CH<sub>2</sub>, 1H), 7.36–7.20 (m, ArH, 35H); <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>) 19.9, 21.9 (CHCH<sub>3</sub>), 68.4 (C-6), 68.9 (C-6'), 73.5, 74.7, 74.8, 74.9, 75.0, 75.1, 75.6 (PhCH<sub>2</sub>), 75.3, 75.9, 77.8, 78.2, 82.1, 82.2, 84.7, 84.8 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 100.3, 101.4 (C-1), 103.8 (C-1'), 114.7, 116.9 (CH=CH<sub>2</sub>), 127.5–128.3 (Ar{C-2, C-3, C-4, C-5, C-6}), 138.5–139.0 (Ar{C-1}), 139.0, 140.1 (CH=CH<sub>2</sub>). β-anomer: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.30 (t, *J* = 4.0 Hz, CHCH<sub>3</sub>, 3H); 3.73–3.32 (m, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', 2 × H-6, 2 × H-6', 12H), 4.26 (t, *J* = 8.0 Hz, H-1', 1H), 4.30 (m, CHCH<sub>3</sub>, 1H), 4.49 (d, *J* = 8.0 Hz, H-1, 1H), 4.98–4.54 (m, 7 × PhCH<sub>2</sub>, 14H), 5.21–5.00 (m, CH=CH<sub>2</sub>, 2H), 5.99–5.72 (m, CH=CH<sub>2</sub>, 1H), 7.36–7.20 (m, ArH, 35H); <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>) 19.9, 21.9 (CHCH<sub>3</sub>), 68.4 (C-6), 68.9 (C-6'), 73.5, 74.7, 74.8, 74.9, 75.0, 75.1, 75.6 (PhCH<sub>2</sub>), 75.3, 75.9, 77.8, 78.2, 82.1, 82.2, 84.7, 84.8 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 100.3, 101.4 (C-1), 103.8 (C-1'), 114.7, 116.9 (CH=CH<sub>2</sub>), 127.5–128.3 (Ar{C-2, C-3, C-4, C-5, C-6}), 138.5–139.0 (Ar{C-1}), 139.0, 140.1 (CH=CH<sub>2</sub>); MS *m/z* 1065 (*M* + *K*, 28%), 1049 (*M* + *Na*, 100), 1025 (*M* – *H*, 38), 523 (*M* – *O*-(3-buten-2-yl)-tri-*O*-benzyl-D-glucopyranosyl, 6), 415 (*M* – *O*-(3-buten-2-yl)-tetra-*O*-benzyl-D-glucopyranosyl, 30); C<sub>65</sub>H<sub>70</sub>O<sub>11</sub>Na requires 1049.4815, found 1049.4794.

Latent/active glycosylation strategies such as this one (and that for *n*-pentenyl glycosides, *vide supra*) are very useful because they can reduce the number of steps required to assemble the blocks needed to construct large oligosaccharides.

For instance, the tetrasaccharide **65** (Scheme 5.17) is built from a single basic donor species **64**, and iterative glycosylation, converting latent to active glycosides at each stage, provides rapid assembly [104]. Without the use of such a latent/active approach, a set of glycosyl donors, differentially protected (i.e.: armed/disarmed) and/or orthogonally substituted at the anomeric position would be required. Boons has also shown that the latent/active approach can be used successfully to build up ‘split and mix’ libraries of both linear [105] and branched oligosaccharides [106], which is a useful application of this technique.



Scheme 5.17

### 5.3.3 Glycosylation reactions with vinyl glycosides: summary

In addition to the work of the Sinaÿ, Chenault and Boons groups in this area, less established methodology employing vinyl glycosides has been reported by Sznaidman *et al.* [107], Marzabadi and Franck [108] and the Takeda group [109]. However, Boons' technique has proven to be the most useful, and the potential of the latent/active strategy for iterative glycosylation and/or library generation makes this a useful method in the general context of oligosaccharide synthesis.

## 5.4 OVERALL SUMMARY

Each of the three glycosylation methodologies that have been considered above has its own place within the toolbox of the synthetic carbohydrate chemist; each has both strengths and weaknesses. The trichloroacetimidate method is undoubtedly the best and most often employed technique for carrying out glycosylation chemistry. However, its one limitation is that it relies on selective deprotection of the anomeric position and successful installation of the imidate when required. *n*-Pentenyl glycosides are useful glycosyl donors in that the *n*-pentenyl group may be carried through many different synthetic manipulations to be activated on demand. In combination with the reactivity tuning and latent/active variants, it is often possible to construct large oligosaccharide targets from a relatively small number of initial monosaccharides. Finally, whilst the latent/active strategy employing vinyl glycosides cannot really compete with the imidate or *n*-pentenyl methods for the

construction of large specific oligosaccharide targets, it finds great applicability in the synthesis of oligosaccharide libraries from small numbers of building blocks. Selection of the correct donor type for any given task is crucial, but given these and the other donors which are available, achievement of a wide variety of synthetic targets is more than feasible.

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## Modern Glycosidation Methods: Tuning of Reactivity

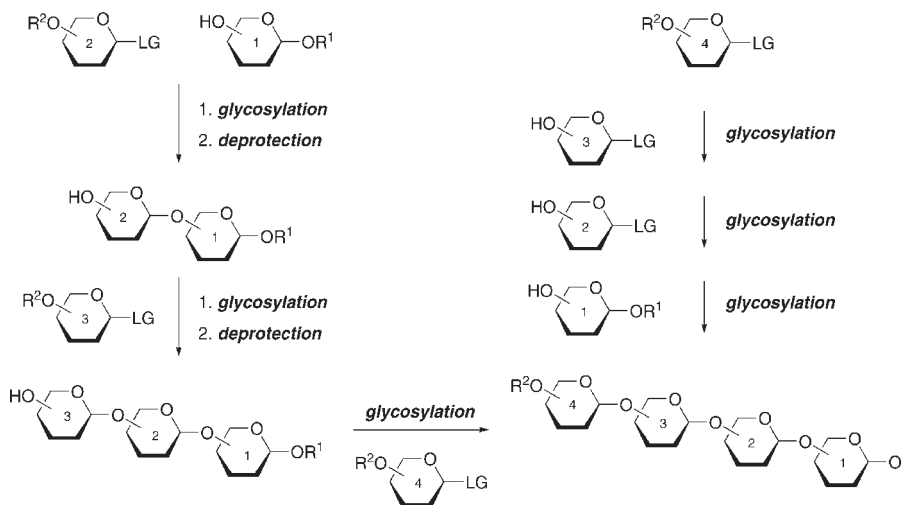
Jörg Pietruszka

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D-70569 Stuttgart, Germany*

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### 6.1 INTRODUCTION

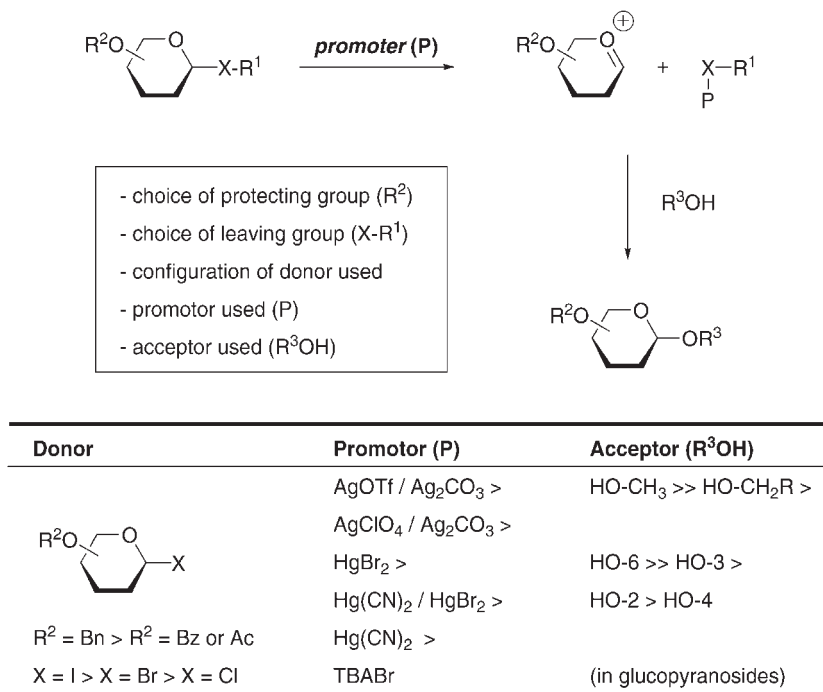
Despite the fact that the number of publications concerned with the chemical synthesis of oligosaccharides has dramatically increased in recent decades, the concise preparation of these complex structures remains a significant challenge. Unlike peptide and nucleotide synthesis, oligosaccharide synthesis is not a facile process. In particular, the regio- and stereocontrolled formation of glycosidic bonds has led to extended synthetic schemes and extensive protecting group manipulations. A typical synthetic approach to (linear) oligosaccharides is shown in Scheme 6.1. Traditionally, the synthesis would start from the reducing end of the oligosaccharide (1). Glycosylation with a fully protected ( $R^2$ ) glycosyl donor (2) gives a disaccharide. After purification and selective deprotection another acceptor is formed, ready for a further coupling step with a fully protected glycosyl donor (3). Repetitive glycosylations and deprotections would ultimately furnish a tetrasaccharide in five steps, assuming no additional protecting group manipulations were necessary. Two different strategies to minimize the number of synthetic steps, and to omit the sometimes tedious unmasking of the hydroxy (acceptor) or anomeric (donor) functionalities have been exploited: ‘Orthogonal Glycosidations’, relying on different leaving groups (LG) in the donor and acceptor, will be discussed in the following chapter (Chapter 7). ‘Tuning of reactivity’ of the glycosyl donors is an alternative approach that is discussed herein. In both cases, and in contrast to the traditional approach, oligosaccharide synthesis starts from the non-reducing end (4) of the target molecule. An ideal strategy for carbohydrate synthesis would involve a consecutive glycosylation–glycosylation–glycosylation sequence, rendering any



**Scheme 6.1** Oligosaccharide assembly: traditional concept (left) and by reactivity tuning of glycosyl donors (right).

protecting group manipulations irrelevant. Moreover, it would be desirable to perform such transformations in one-pot. It has recently been demonstrated that these aims can indeed be realised by carefully manipulating the reactivity of the donors used in the synthesis. Theoretically, synthesis of the depicted tetrasaccharide would require hermaphrodites (molecules with the potential to react as both donors and acceptors) offering four different levels of reactivity (4)–(1) (in decreasing reactivity). The aim of this chapter is to describe the different possibilities for tuning the reactivities of the glycosyl donors and hermaphrodites.

Although Glaudemans and Fletcher showed as early as 1965 that the rate of solvolysis of glycofuranosyl halides strongly depended on the protecting group pattern [1], the strategic foundations for a convergent synthetic route to oligosaccharides were laid by Paulsen more than a decade later [2]. He noted a considerable influence on the reactivity of the donor by the LG and the protecting groups, as well as a dependence on the rate of the glycosylation on both the promotor and the glycosyl acceptor employed (Scheme 6.2). Whereas the reactivity of the alcohol and the influence of the configuration of either donor and acceptor is difficult to assess and adjust, some decisive factors for mediating the reactivities of the donors can be more easily illustrated. Without taking solvent effects into account, the first step of the glycosylation process, the formation of a cyclic oxonium ion by loss of an LG from the donor, will determine the outcome of competing glycosylations. Torsional and electronic effects of the protecting groups can decrease the rate of oxonium ion formation, and hence glycosylation, by destabilizing the putative intermediate. The choice of the LG is also critical: again, activation by the promotor can be enhanced or disfavoured by altering electronic or steric factors.

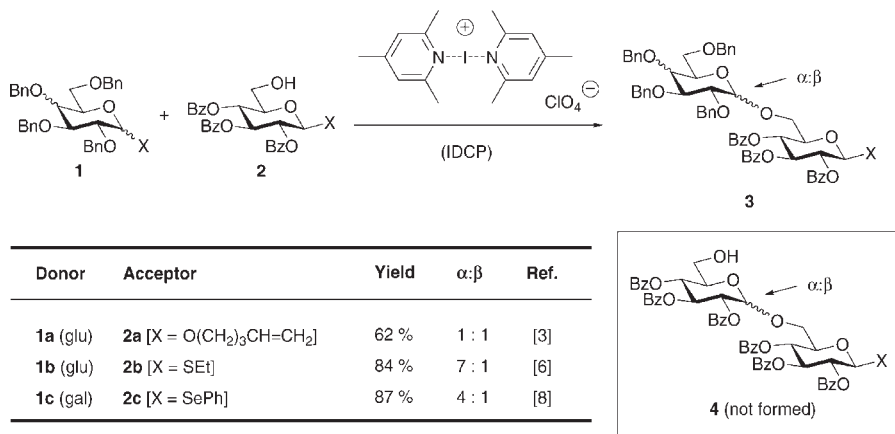


**Scheme 6.2** Tuning the reactivity of glycosylations; below: relative reactivities of glycosyl halides [2].

## 6.2 INFLUENCE OF PROTECTING GROUPS

### 6.2.1 Electronic effects

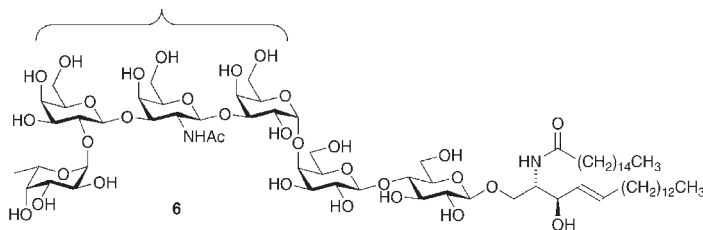
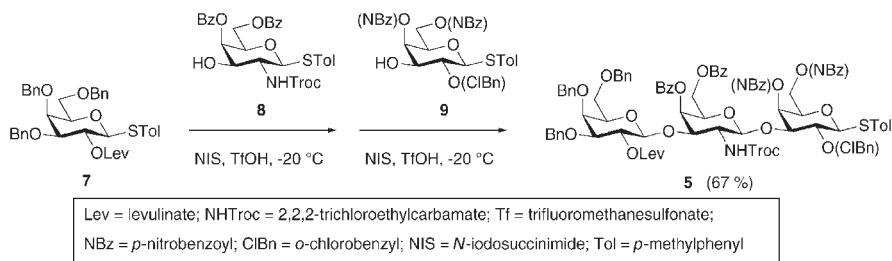
Although the ability to mediate the reactivities of glycosyl donors and acceptors was noted relatively early, it was not strategically used for the selective activation of one donor in the presence of a second (that could potentially be activated by the same promotor) until 1988. Fraser-Reid's group reported the ability of *n*-pentenyl glycosides to act as stable glycosyl donors and disclosed the armed/disarmed or reactivity tuning concept (see also Chapter 5) [3–5]. He found that oxidative hydrolysis of the *n*-pentenyl glycoside with *N*-bromosuccinimide (NBS) in water required minutes when the C-2 protecting group was an ether (e.g. benzyl, Bn), but hours when an ester (e.g. benzoyl, Bz, or acetate, Ac) was used. This observation led to the first successful couplings of armed (activated) donors with partially protected disarmed (deactivated) acceptors: selective activation of the more reactive donor **1a** with 1 equiv. of the promotor (IDCP: iodonium dicollidine perchlorate) in the presence of the deactivated pentenyl glycoside **2a** furnished disaccharide **3** as a



**Scheme 6.3** Tuning the reactivity of glycosylations: electronic effect of protecting groups.

1:1 mixture of anomers in good yield. However, no self-coupling product **4** was formed (Scheme 6.3). This observation can be explained either by a reversible and rapid (as compared to the subsequent steps) activation of the glycosyl donor with the promotor or by a directed transfer of the promoting agent between activated and unactivated donors in a bimolecular reaction. It is important to note that the disaccharide **3** thus obtained is now available for further coupling with a more reactive promotor. The reactivity tuning concept is not limited to *n*-pentenyl glycosides, but can also be extended to include thio- [6, 7] and selenoglycosides [8, 9] **1b–c/2b–c**. As a rewarding side effect, the  $\alpha/\beta$  selectivities of the latter reactions were also improved (Scheme 6.3). Chemoselective activation was also extended, for instance, to glycosyl fluorides [10] and phosphoroamidates [11]. Moreover, Danishefsky *et al.* were the first to incorporate glycals within reactivity tuning strategies [12, 13].

In order to extend the concept to allow the rapid assembly of larger systems, more levels of reactivity were required. This would afford the opportunity for directly preparing oligosaccharides in a one-pot fashion without the need for protecting-group manipulations, thus considerably reducing the number of steps conventionally needed. Extensive work on quantifying glycosyl donor reactivity was first reported by Ley *et al.* [14] and later by Wong *et al.* [15, 16] Detailed competition studies led to a better understanding of the influence of individual protecting groups, in certain positions of a given monosaccharide unit, on reactivity levels. Nowadays the obtained data can be used as a crude predictive tool to design oligosaccharide syntheses. Focusing on electronic effects, it was demonstrated that the deactivating power decreases in the following order: N<sub>3</sub> > O(Cl)Ac ( $\alpha$ -chloroacetate) > NPhth (phthalimido) > OBz > OAc > NHTroc > OBn > OH > OSilyl > H. The position of the substituent is also essential and even remote groups can have a profound

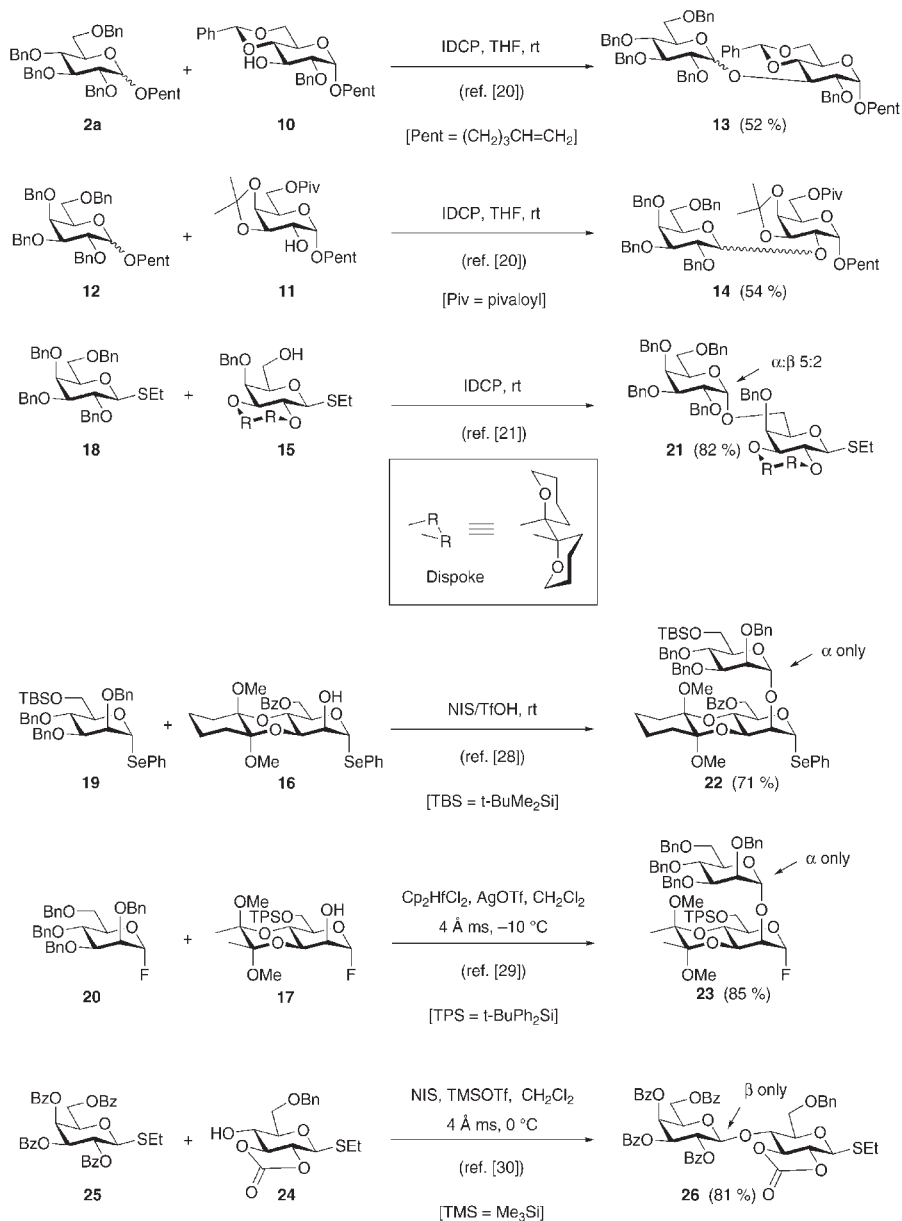


**Scheme 6.4** One-pot synthesis of the key trisaccharide **5** of the Globo H glycolipid **6** [15].

influence on the outcome of a one-pot sequence. The precise effect is not always easy to predict: Whereas the observed influence of the position of the benzoate group is  $4 > 3 > 2 > 6$  for galactose, it is different for the mannose system ( $2 > 6 > 4 > 3$ ). In cases where high  $\alpha/\beta$  ratios were expected, the knowledge of these facts led to a variety of efficient one-pot trisaccharide syntheses, for example, entry to the key trisaccharide **5** of the Globo H glycolipid **6** (Scheme 6.4) [17], isolated and identified as an antigen on prostate and breast cancer cells, has been reported [18, 19]. Activated donor **7** ( $\beta$ -selectivity controlled by neighbouring group participating temporary protecting group on the 2-position) was reacted with the slightly deactivated (approx. factor 4.7) thioglycoside **8**. The intermediate disaccharide was not isolated, but directly treated with *N*-iodosuccinimide (NIS) in the presence of compound **9**, furnishing the target molecule **5** (67%). Fine-tuning was achieved by the beneficial use of specific electron-withdrawing groups (benzoyl, *p*-nitrobenzoyl, and *o*-chlorobenzyl ethers).

### 6.2.2 Torsional effects

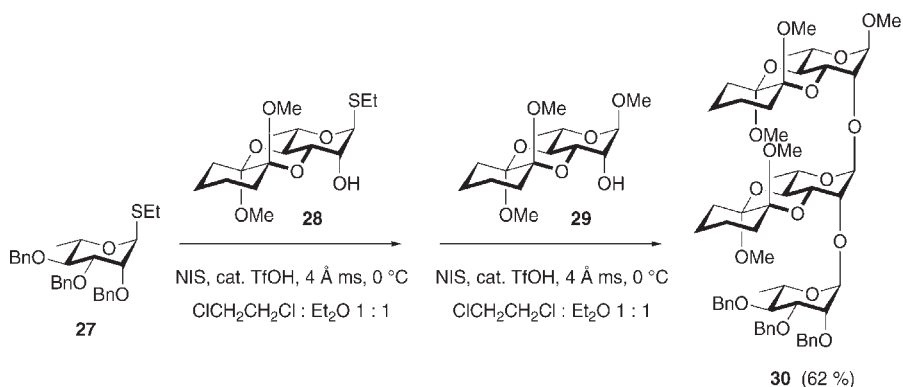
Cyclic protecting groups have also been shown to deactivate the anomeric LG (Scheme 6.5) and this has been rationalized in terms of the torsional strain that is created upon the formation of the cyclic oxonium ion: the process of flattening the  $^4C_1$  chairs in pyranosides is especially disfavoured for conformationally restrained, bicyclic glycosyl donors. First examples included glycosides bearing a variety of cyclic acetals as protecting groups, e.g. the 1,3-dioxane or 1,3-dioxolane derivatives



**Scheme 6.5** Tuning the reactivity of glycosyl donors: torsional effect of the protecting group.

**10** and **11** (Scheme 6.5). Perbenzylated donors **2a** and **12** were selectively activated and the disaccharides **13** and **14** formed in good yield. In general, it was found that 2,3- or 3,4-isopropylidene protected sugars were more deactivated than the corresponding 4,6-benzylidene acetals or related 1,3-dioxanes [20]. With the discovery of the dispiroketal (dispoke) [21, 22], cyclohexane-1,2-diacetal (CDA) [23, 24], and later butane-3,4-diacetal (BDA) protecting groups [25, 26], it was possible to further extend this strategy to larger systems [27]. Again, deactivation was observed with thioglycosides (e.g. **15**) [21], selenoglycosides (e.g. **16**) [28], and glycosyl fluorides (e.g. **17**) [29], relative to the highly reactive donors **18–20**. The disaccharides **21–23** were obtained in high yield. Obviously, another reactivity level was introduced; the reactivity differences between these systems are slight, however, systematic investigations indicate that the deactivating effects decrease in the order BDA > dispoke > CDA [14]. Zhu and Boons introduced *trans*-2,3-cyclic carbonates such as glycoside **24**, having a significantly lower anomeric reactivity than the corresponding fully acylated donor **25**. The resulting disaccharide **26** can be further activated to give predominantly 1,2-*cis*-linked trisaccharides [30].

The high potential of CDA-protected glycosides in one-pot oligosaccharide synthesis was first demonstrated by Ley and Priepke in 1994 [31]. The trisaccharide unit found in the common polysaccharide-antigen Group B *Streptococci* was obtained from suitable protected rhamnoside precursors **27–29**: Selective activation of fully benzylated thioglycoside **27** in the presence of the CDA protected rhamnoside **28** gave a disaccharide which again was not isolated (Scheme 6.6). Alcohol **29** and a second equivalent of NIS/TfOH were added. Trirhamnoside **30** was obtained in 62% yield and readily deprotected in two consecutive steps (not shown).



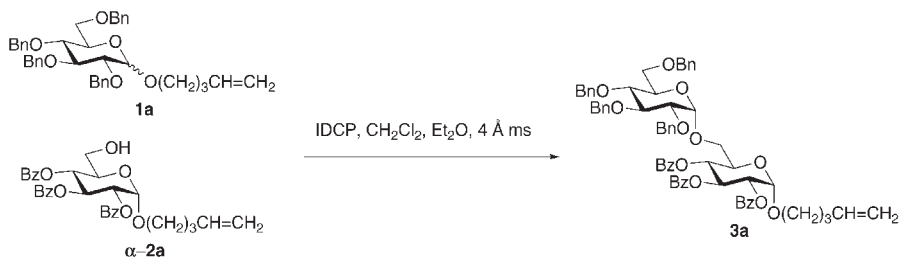
**Scheme 6.6** One-pot synthesis of trisaccharide **30** [31].



### 6.2.3 Experimental details

#### Method 1

Synthesis of Pent-4-enyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)- $\alpha$ -D-glucopyranoside (**3**) [32].



*Notes and discussion.* Although it had been well documented that protecting groups could influence the reactivity of glycosyl donors [1, 2], Fraser-Reid *et al.* demonstrated for the first time that an activated pentenyl donor **1a** could be selectively activated in the presence of a second, deactivated, pentenyl acceptor **2a**. The disaccharide **3a** thus obtained could be further activated to allow formation of a glycopeptide [32].

#### Materials.

Pent-4-en-1-yl 2,3,4-tri- <i>O</i> -benzoyl- $\alpha$ -D-glucopyranoside ( $\alpha$ - <b>2a</b> ) [32] (576 mg, 1.03 mmol)	assume toxic
Pent-4-en-1-yl 2,3,4,6-tetra- <i>O</i> -benzyl- $\alpha$ / $\beta$ -D-glucopyranoside ( <b>1a</b> ) [5] (762 mg, 1.25 mmol)	assume toxic
IDCP (773 mg, 1.65 mmol)	assume toxic
4 Å molecular sieves (2 g preactivated powder)	
Dry dichloromethane (7.5 ml; distilled from calcium hydride)	toxic, irritant
Dry diethyl ether (30 ml; distilled from sodium benzophenone ketyl)	extremely flammable, harmful
10% sodium thiosulfate solution (10 ml)	irritant
Chloroform (400 ml)	irritant, danger of serious (irreversible) damage to health
1% aqueous hydrochloric acid (100 ml)	irritant
10% aqueous sodium bicarbonate solution (100 ml)	
Magnesium sulfate	

*Equipment.*

Round bottomed flask (100 ml) with stirrer bar and septum (pressure balance!)  
Magnetic stirrer  
Silver foil  
Syringes  
Measuring cylinder  
Funnel  
Separating funnel  
Conical flasks (250 and 500 ml)  
Round bottomed flask (1000 ml)  
Rotary evaporator  
Flash column chromatography equipment

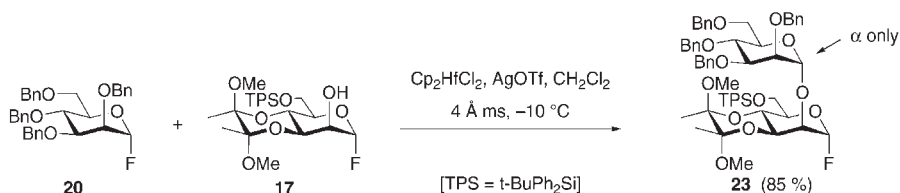
*Special precautions.* The reaction is carried out under argon in predried glassware; the reaction vessel should be wrapped in silver foil. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place pent-4-en-1-yl 2,3,4-tri-*O*-benzoyl- $\alpha$ -D-glucopyranoside ( **$\alpha$ -2a**) (576 mg, 1.03 mmol), 4 Å molecular sieves (2 g), and IDCP (773 mg, 1.65 mmol) in a predried round bottomed flask that is wrapped in silver foil. Evacuate the flask repeatedly and flush with argon, before adding a solution of pent-4-en-1-yl 2,3,4,6-tetra-*O*-benzyl- $\alpha$ /β-D-glucopyranoside (**1a**) (762 mg, 1.25 mmol) in dry diethyl ether/CH<sub>2</sub>Cl<sub>2</sub> (37.5 ml of a 4:1 mixture). Stir the mixture at room temperature under argon for 10 h. Add 10% aqueous thiosulfate solution (10 ml), filter the mixture, and wash the collected sieves with chloroform (100 ml). Separate the organic layer, wash with cold (0 °C) 1% aqueous hydrochloric acid (100 ml) and extract the aqueous phase with chloroform (3 × 100 ml). Wash the combined organic extracts with cold (0 °C) 10% sodium bicarbonate solution (100 ml), dry over magnesium sulfate, filter, and remove the solvents under reduced pressure. Purify the residue by flash column chromatography (eluent: petroleum ether 40/60: ethyl acetate 9:1, then 4:1) to give slightly impure disaccharide **3a**. The product is obtained after further chromatographic purification (eluent: chloroform stabilized with 0.75% ethanol), yielding 701 mg (0.65 mmol, 63%); [ $\alpha$ ]<sub>D</sub><sup>22</sup> = +69.0 (*c* 0.83 in CHCl<sub>3</sub>), <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 7.99 (2H, d, *J* = 8.8 Hz, Ar-H), 7.96 (2H, dd, *J* = 8.7 Hz, 1.1, Ar-H), 7.87 (2H, dd, *J* = 7.7 Hz, 1.2, Ar-H), 7.53–7.11 (29H, m, Ar-H), 6.16 (1H, t, *J* = 9.8 Hz, 3-H), 5.68 (1H, ddt, *J* = 17.9, 9.3, 6.5 Hz, CH=CH<sub>2</sub>), 5.54 (1H, t, *J* = 9.9 Hz, 4-H), 5.30 (1H, d, *J* = 3.7 Hz, 1-H), 5.23 (1H, dd, *J* = 10.2, 3.8 Hz, 2-H), 4.94–4.34 (11H, m, 4 × CH<sub>2</sub>Ph, CH=CH<sub>2</sub>, 1'-H), 3.97 (1H, t, *J* = 9.3 Hz, 3'-H), 3.88–3.38 (10H, m, 5-H, 6-H<sub>a/b</sub>, 2'-H, 4'-H, 5'-H, 6'-H<sub>a/b</sub>, OCH<sub>2</sub>CH<sub>2</sub>), 2.08–2.01 (2H, m, CH<sub>2</sub>CH=CH<sub>2</sub>), 1.69–1.61 (2H, m, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

### Method 2

Synthesis of (2'*S*,3'*S*)-6-*O*-*tert*-butyldiphenylsilyl-3-*O*,4-*O*-[2',3'-dimethoxybutan-2',3'-diyl]-2-*O*-(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-mannopyranosyl)- $\alpha$ -D-mannopyranosyl fluoride (**23**) [29].



**Notes and discussion.** Glycosyl fluorides have proved excellent donors in oligosaccharide synthesis [33, 34]. A first application of reactivity tuning was communicated by Castillon *et al.* using the electronic difference of the donors [10]. Torsional effects were investigated and beneficially applied in oligosaccharide synthesis by Ley *et al.* [29, 35]. The high  $\alpha$ -selectivity (no  $\beta$ -product reported) in combination with the possibility of using glycosyl fluorides in orthogonal glycosylation strategies (see Chapter 7) makes this approach especially versatile for rapid, one-pot synthesis of linear oligosaccharides.

### Materials.

2,3,4,6-Tetra-*O*-benzyl- $\alpha$ -D-mannopyranosyl fluoride (**20**) assume toxic  
[29] (112 mg, 0.21 mmol)

(2'*S*,3'*S*)-6-*O*-*tert*-Butyldiphenylsilyl-3-*O*,4-*O*-[2',3'-dimethoxybutan-2',3'-diyl]- $\alpha$ -D-mannopyranosyl fluoride (**17**) [29] (100 mg, 0.19 mmol) assume toxic

Silver trifluoromethanesulfonate (135 mg, 0.51 mmol; dried azeotropically with toluene and *in vacuo* before use) irritant

Hafnocene dichloride (78.0 mg, 0.21 mmol) irritant

4 Å Molecular sieves (1 g preactivated powder)

Dry dichloromethane (5 ml; distilled from calcium hydride) toxic, irritant

Celite<sup>®</sup>

Dichloromethane (60 ml) toxic, irritant

Saturated sodium bicarbonate solution (25 ml)

Magnesium sulfate

### Equipment.

Round bottomed flasks (10 and 25 ml) with stirrer bars and septa (pressure balance!)

Magnetic stirrer

Ice-salt bath with thermometer  
Syringes  
Measuring cylinder  
Funnel  
Separating funnel  
Conical flasks (100 ml)  
Round bottomed flask (100 ml)  
Rotary evaporator  
Flash column chromatography equipment

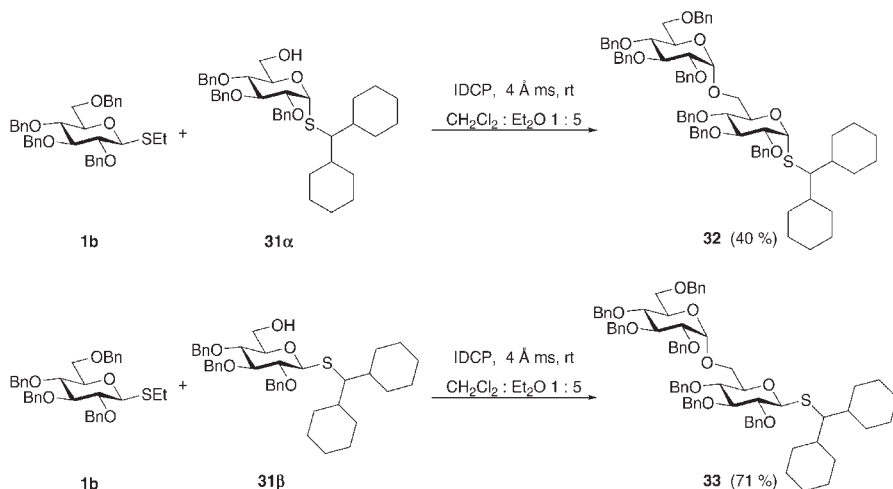
*Special precautions.* The reaction is carried out under argon in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place AgOTf (135 mg, 0.51 mmol), 4 Å molecular sieves (1.00 g) and Cp<sub>2</sub>HfCl<sub>2</sub> (78.0 mg, 0.21 mmol) into a predried round bottomed flask. After repeated evacuation and flushing with argon, suspend the mixture in CH<sub>2</sub>Cl<sub>2</sub> (2.00 ml), cool to –10 °C and stir for 5 min. Place in a second predried flask glycosyl fluorides **20** (112 mg, 0.21 mmol) and **17** (100 mg, 0.19 mmol). After evacuation and flushing with argon, add CH<sub>2</sub>Cl<sub>2</sub> (3 ml). Add this solution via syringe to the suspension containing the mixture of catalysts. Continue to stir at –10 °C for 20 min and then filter the contents of this flask through Celite<sup>®</sup>. Wash the filter-cake with CH<sub>2</sub>Cl<sub>2</sub> (40 ml) and the combined filtrates with aqueous saturated sodium bicarbonate solution (25 ml). Extract the aqueous layer with CH<sub>2</sub>Cl<sub>2</sub> (20 ml) and dry the combined organic extracts (MgSO<sub>4</sub>), filter and concentrate *in vacuo* to obtain an oil. Purification by flash column chromatography (eluent: petroleum ether 40/60:diethyl ether 7:3) furnishes the dimannoside **23** (169 mg, 0.16 mmol, 85%) as a foam; (600 MHz, CDCl<sub>3</sub>) 7.72–7.13 (30H, m, Ar-H), 5.62 (1H, d, *J* = 47.3 Hz, 1-H), 5.58 (1H, s, 1'-H), 4.84 (1H, d, *J* = 10.6 Hz, PhCH<sub>a</sub>H<sub>b</sub>-A), 4.82 (1H, d, *J* = 12.2 Hz, PhCH<sub>a</sub>H<sub>b</sub>-B), 4.70 (1H, d, *J* = 12.1 Hz, PhCH<sub>a</sub>H<sub>b</sub>-C), 4.59 (1H, d, *J* = 12.5 Hz, PhCH<sub>a</sub>H<sub>b</sub>-B), 4.56 (1H, d, *J* = 12.1 Hz, PhCH<sub>a</sub>H<sub>b</sub>-C), 4.47–4.43 (2H, m, PhCH<sub>a</sub>H<sub>b</sub>-A, PhCH<sub>a</sub>H<sub>b</sub>-D), 4.37 (1H, d, *J* = 11.4 Hz, PhCH<sub>a</sub>H<sub>b</sub>-D), 4.35 (1H, t, *J* = 10.0 Hz, 4-H), 4.22 (1H, s, 2-H), 4.11 (1H, d, *J* = 10.5 Hz, 3-H), 3.99–3.96 (3H, m, 2'-H, 4'-H, 6-H<sub>a</sub>), 3.91–3.87 (2H, m, 3'-H, 5-H), 3.85–3.80 (3H, m, 6-H<sub>b</sub>, 5'-H, 6'-H<sub>a</sub>), 3.75 (1H, d, *J* = 8.8 Hz, 6'-H<sub>b</sub>), 3.29 (3H, s, OCH<sub>3</sub> BDA), 3.24 (3H, s, OCH<sub>3</sub> BDA), 1.32 (3H, s, CH<sub>3</sub> BDA), 1.31 (3H, s, CH<sub>3</sub> BDA), 0.99 (9H, s, SiC(CH<sub>3</sub>)<sub>3</sub>).

### 6.3 INFLUENCE OF LEAVING GROUP

While the preceeding section discussed the influence of protecting groups on the relative reactivity of glycosyl donors, the present section deals with the possibility of tuning the reactivity by incorporating different anomeric LGs. The same promotor is used for both the donor and acceptor. Thus, additional distinct levels of anomeric reactivity of glycosides are achieved.



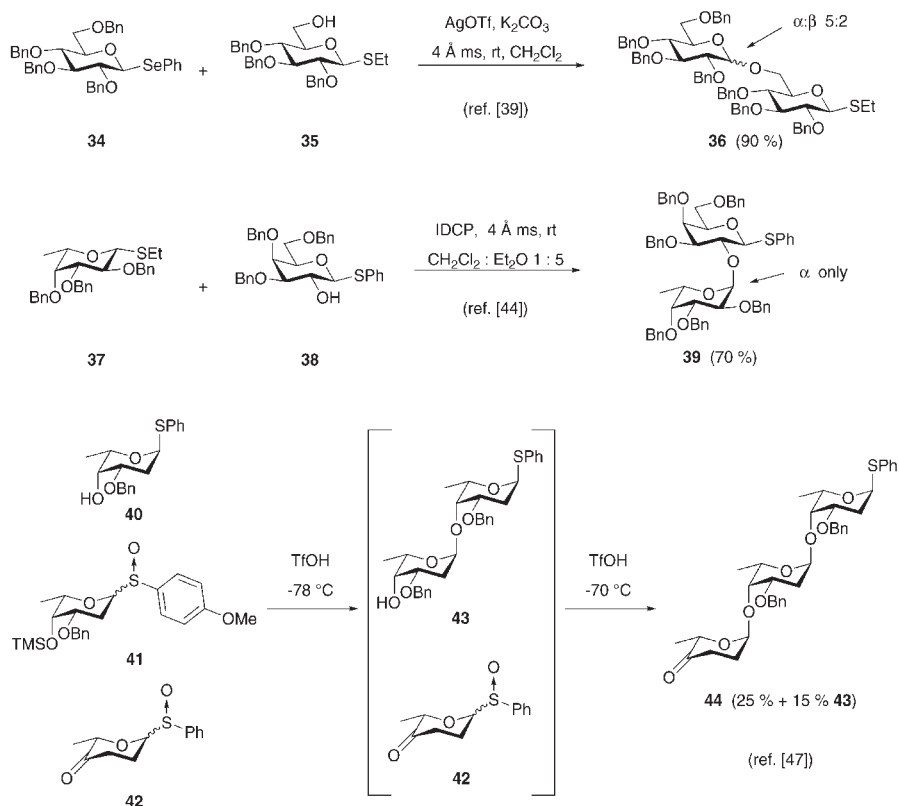
**Scheme 6.7** Tuning the reactivity of glycosylations: steric effect of LG [37].

### 6.3.1 Steric effects

There are few reports on the concept of tuning the reactivity of the glycosyl donor by altering the bulk of the anomeric group. Boons *et al.* were the first to describe the influence of steric effects, within the anomeric thiol group, on glycosyl reactivity [36, 37]. Using the same protecting groups for the donor and acceptor, it was noted that ethyl thioglycosides (e.g. **1b**) were selectively activated by IDCP in the presence of dicyclohexylmethyl thioglycosides (e.g. **31**). The anomeric configuration also had a pronounced effect: Compared with the  $\beta$ -anomer **31 $\beta$** , the  $\alpha$ -anomer **31 $\alpha$**  was generally less readily glycosylated (Scheme 6.7). As expected, further glycosidations could proceed with the sterically deactivated donors when an electronically deactivated (protecting groups) acceptor was present, hence disaccharides **32** or **33** could be directly used for trisaccharide synthesis.

### 6.3.2 Electronic effects

Electronically different LGs have also been incorporated to tune the glycosyl donor reactivity (Scheme 6.8). One of the first examples described the use of selenoglycosids in the presence of thioglycosides. While Mehta and Pinto used silver triflate to selectively activate the seleno derivative **34**—the benzylated sulfur compound **35** did not react—for the synthesis of disaccharide **36** [38, 39], iodonium sources became more common for numerous applications of this concept in one-pot



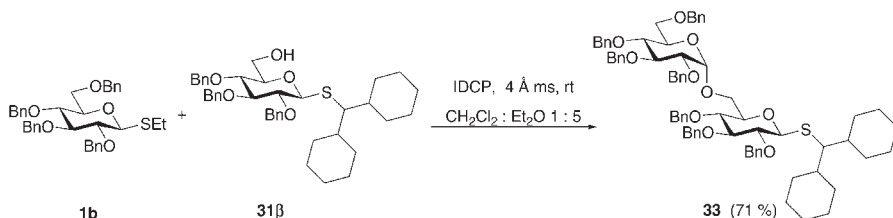
**Scheme 6.8** Tuning the reactivity of glycosylations: electronic effects of LG.

oligosaccharide assemblies [28, 40–43]. Aryl groups can also be profitably used: the observed chemoselectivity for the transformation of glycosides **37** and **38** to disaccharide **39** [44] (also an intermediate for the synthesis of the tumor-associated antigen Globo-H) was based not only on the fact that 6-deoxy-sugars are generally more reactive than their 6-hydroxy counterparts, but also on the considerably higher reactivity of ethyl over phenyl thioglycosides [45, 46]. Moreover, by attaching additional substituents to the phenyl ring, the glycoside reactivity can be further modified. This resulted in the first one-pot synthesis of the ciclamycin trisaccharide by Raghavan and Kahne in 1993 [47]. Whereas, in the presence of triflic acid, the anomeric sulfide **40** can only act as an acceptor, sulfoxides **41** and **42** can act as donors. In the competing step, the methoxy derivative **41** reacted considerably faster to form disaccharide **43** (after cleavage of the silyl ether). Upon warming to  $-70\text{ }^\circ\text{C}$ , the less reactive donor **42** was activated forming the target molecule **44**. Upon oxidation this could be directly coupled with the required aglycon.

### 6.3.3 Experimental details

#### Method 3

Synthesis of Dicyclohexylmethyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,5-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-1-thio- $\beta$ -D-glucopyranoside (**33**) [37].



*Notes and discussion.* Boons *et al.* based their chemoselective glycosylation strategy on the fact that the steric bulk of the anomeric thiol group could control the reactivity of the glycosyl donor. It is worth mentioning that the yield of this transformation strongly depended on the configuration of the anomeric center of the acceptor. Disaccharide **33** and related compounds could be further activated in the presence of less reactive acceptors yielding the corresponding trisaccharides [36, 37].

#### Materials.

Ethyl 2,3,4,6-tetra- <i>O</i> -benzyl-1-thio- $\beta$ -D-glucopyranoside ( <b>1b</b> ) [48] (121 mg, 0.21 mmol)	assume toxic
Dicyclohexylmethyl 2,3,4-tri- <i>O</i> -benzyl-1-thio- $\beta$ -D-glucopyranoside ( <b>31</b> $\beta$ ) [37] (105 mg, 0.16 mmol)	assume toxic
IDCP (226 mg, 0.48 mmol)	assume toxic
4 Å molecular sieves (500 mg preactivated powder)	
Dry dichloromethane (0.5 ml; distilled from calcium hydride)	toxic, irritant
Dry diethyl ether (2.5 ml; distilled from sodium benzophenone ketyl)	extremely flammable, harmful
Dichloromethane (75 ml)	toxic, irritant
15% sodium thiosulfate solution (30 ml)	irritant
Magnesium sulfate	

#### Equipment.

- Round bottomed flask (10 ml) with stirrer bar and septum (pressure balance!)
- Magnetic stirrer
- Measuring cylinder

Funnel  
 Separating funnel  
 Conical flasks (100 ml)  
 Round bottomed flask (250 ml)  
 Rotary evaporator  
 Chromatography equipment

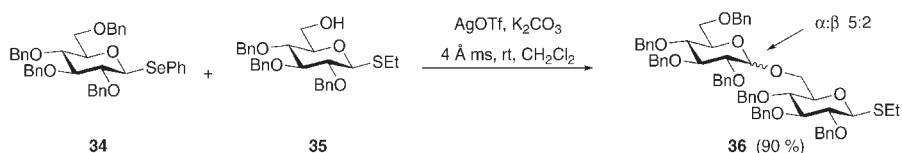
*Special precautions.* The reaction is carried out under argon in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio- $\beta$ -D-glucopyranoside (**1b**) [48] (121 mg, 0.21 mmol), dicyclohexylmethyl 2,3,4-tri-*O*-benzyl-1-thio- $\beta$ -D-glucopyranoside (**31 $\beta$** ) [37] (105 mg, 0.16 mmol), and powdered 4 Å molecular sieves (500 mg) into a round bottomed flask and repeatedly evacuate and flush with argon. Add dry Et<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub> (3 ml of a 5:1 mixture), followed by IDCP (226 mg, 0.48 mmol) and continue to stir for 2 h at room temperature. Filter the reaction mixture, dilute with CH<sub>2</sub>Cl<sub>2</sub> (75 ml) and transfer to a separatory funnel. Wash the organic layer with a 15% aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution (2 × 15 ml) and water (2 × 20 ml), dry over MgSO<sub>4</sub>, filter, and concentrate under reduced pressure. Size exclusion column chromatography of the residue (LH-20; eluent: CH<sub>2</sub>Cl<sub>2</sub>/MeOH 1:1) yields the product **33** as a colorless syrup (133 mg, 0.11 mmol, 71%). Alternatively, silica gel column chromatography can be applied for purification (eluent: CH<sub>2</sub>Cl<sub>2</sub>/acetone 996:4). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +3.3 (*c* 1.0), <sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.45–7.15 (35H, m, Ar-H), 5.20 (1H, d, *J* = 3.5 Hz, 1'-H), 4.99–4.46 (14H, m, 7 × CH<sub>2</sub>Ph), 4.34 (1H, d, *J* = 9.7 Hz, 1-H), 3.97 (1H, t, *J* = 8.9 Hz, 3'-H), 3.88 (1H, dd, *J* = 12.5, 3.5 Hz, 6'-H<sub>a</sub>), 3.81–3.76 (m, 3H, 5-H, 4'-H, 6'-H<sub>b</sub>), 3.70 (1H, dd, *J* = 10.8, 3.8 Hz, 6-H<sub>a</sub>), 3.65–3.56 (4H, m, 2-H, 4-H, 6-H<sub>b</sub>, 3'-H), 3.32 (1H, m, 5'-H), 3.11 (1H, dd, 2'-H), 2.37 (1H, m, CHS), 1.97 and 1.80–1.00 (22H, m, 2 × C<sub>6</sub>H<sub>11</sub>).

#### Method 4

Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl- $\alpha/\beta$ -D-glucopyranosyl)-1-thio- $\beta$ -D-glucopyranoside (**36**) [39].



*Notes and discussion.* Selenoglycosides are well known as versatile glycosyl donors and acceptors [8, 9, 38, 39], and have played crucial roles in oligosaccharide assembly (*vide infra*). In particular, the preferential activation or deactivation of these substrates relative to other glycosyl-moieties has been exploited [28, 35, 39, 42].



*Materials.*

Phenyl 2,3,4,6-tetra- <i>O</i> -benzyl-1-seleno- $\beta$ -D-glucopyranoside ( <b>34</b> ) [39] (170 mg, 0.25 mmol)	assume toxic
Ethyl 2,3,4-tri- <i>O</i> -benzyl-1-thio- $\beta$ -D-glucopyranoside ( <b>35</b> ) [49] (124 mg, 0.25 mmol)	assume toxic
Silver trifluoromethanesulfonate (385 mg, 1.50 mmol; dried azeotropically with toluene and <i>in vacuo</i> before use)	irritant
Anhydrous potassium carbonate (1.04 g, 7.50 mmol)	
4 Å molecular sieves (1 g preactivated powder)	
Dry dichloromethane (8 ml; distilled from calcium hydride)	toxic, irritant
Celite <sup>®</sup>	
Dichloromethane (20 ml)	toxic, irritant

*Equipment.*

Round bottomed *Schlenk*-flask (25 ml) with stirrer bar and septum (pressure balance!)

Magnetic stirrer

Syringe

Measuring cylinder

Funnel

Separating funnel

Conical flasks (100 ml)

Round bottomed flask (50 ml)

Rotary evaporator

Flash column chromatography equipment

*Special precautions.* The reaction is carried out under nitrogen in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Dry a mixture of phenyl 2,3,4,6-tetra-*O*-benzyl-1-seleno- $\beta$ -D-glucopyranoside (**34**) [39] (170 mg, 0.25 mmol), ethyl 2,3,4-tri-*O*-benzyl-1-thio- $\beta$ -D-glucopyranoside (**35**) [49] (124 mg, 0.25 mmol), and 4 Å molecular sieves (1 g) under vacuum overnight. After addition of anhydrous CH<sub>2</sub>Cl<sub>2</sub> (8 ml), stir the reaction mixture under an atmosphere of nitrogen for 1 h. Add dry K<sub>2</sub>CO<sub>3</sub> (1.04 g, 7.50 mmol) and AgOTf (385 mg, 1.50 mmol). After tlc indicates completion of reaction (approx. 1.5 h), filter the mixture through a pad of Celite<sup>®</sup> flushing with CH<sub>2</sub>Cl<sub>2</sub>. Wash the organic layer with water (2 × 10 ml), dry over MgSO<sub>4</sub>, filter, and concentrate under

reduced pressure. Purify the residue by column chromatography (eluent: hexane/ethyl acetate 4:1) to obtain a 5:2  $\alpha/\beta$  mixture of product **36** (229 mg, 0.23 mmol, 90%);  $\alpha$ -isomer:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ): 7.50–7.10 (35H, m, Ar-H), 5.07 (1H, d,  $J = 2.3$  Hz, 1'-H), 4.98 (1H, d,  $J = 10.8$  Hz,  $\text{CH}_a\text{H}_b\text{Ph}$ ), 4.71–4.92 (8H, m,  $3 \times \text{CH}_a\text{H}_b\text{Ph}$ ,  $5 \times \text{CH}_a\text{H}_b\text{Ph}$ ), 4.67 (1H, d,  $J = 11.0$  Hz,  $\text{CH}_a\text{H}_b\text{Ph}$ ), 4.63 (1H, d,  $J = 12.0$  Hz,  $\text{CH}_a\text{H}_b\text{Ph}$ ), 4.57 (1H,  $J = 10.2$  Hz,  $\text{CH}_a\text{H}_b\text{Ph}$ ), 4.42–4.50 (3H, m, 1-H,  $2 \times \text{CH}_a\text{H}_b\text{Ph}$ ), 3.98 (1H, t,  $J = 18.6$  Hz, 3'-H), 3.86 (1H, m, 5'-H), 3.74–3.60 (5H, m, 3-H, 4-H, 4'-H, 6'-H<sub>a</sub>, 6'-H<sub>b</sub>), 3.59 (1H, dd,  $J = 9.7, 3.3$  Hz, 2'-H), 3.48 (1H, m, 5-H), 3.19 (1H, t,  $J = 18.2$  Hz, 2-H), 2.69 (2H, m,  $\text{SCH}_2\text{CH}_3$ ), 1.26 (3H, t,  $\text{SCH}_2\text{CH}_3$ ).

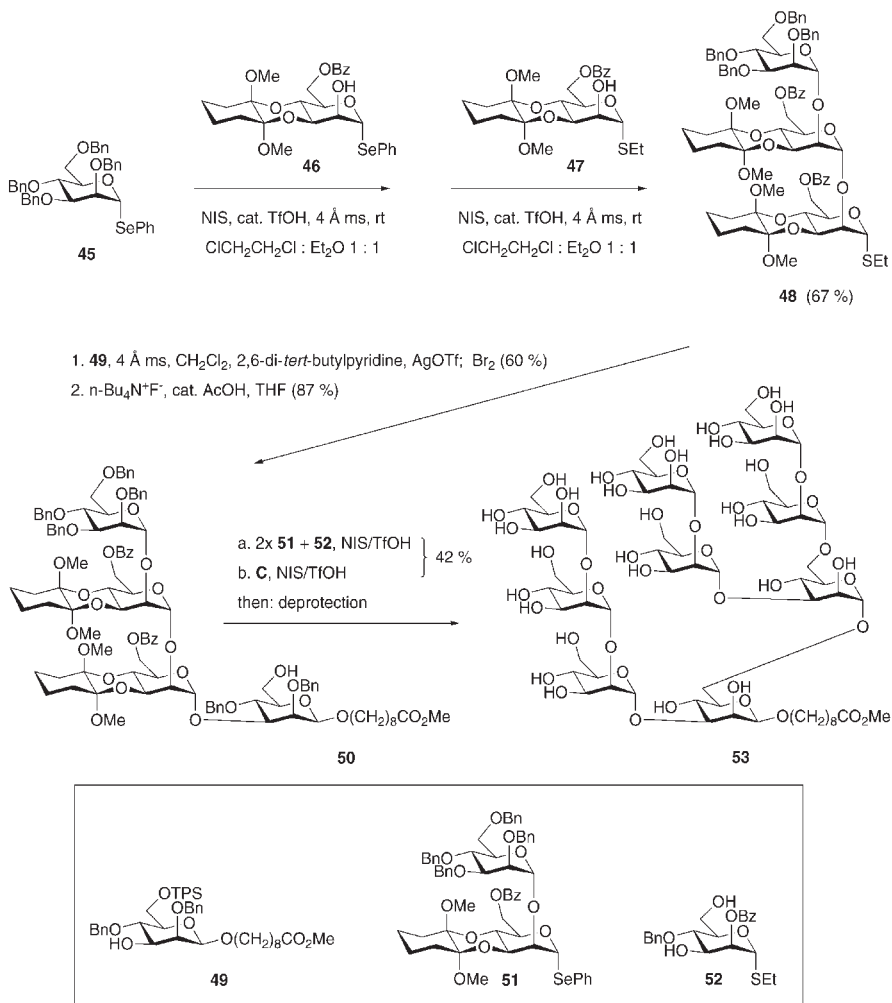
## 6.4 SUMMARY

Chemoselective strategies, relying on one-pot sequential glycosylations, can greatly facilitate the rapid synthesis of oligosaccharides. The basis for a programmed, predictable design is the knowledge of the relative reactivity of glycosyl donors; ways of introducing and fine-tuning different reactivity levels should be appreciated. The systematic investigations to quantify donor reactivities by Ley *et al* [14], and Wong *et al.* [15], provide excellent leads: For a given LG, reliable predictions of the reactivity as a function of protecting group patterns are possible. In addition, it has been established that the carbohydrate configuration is also important (reactivity decreases in the following order: fucose > galactose > glucose > mannose), as well as the fact that glycosylated products are slightly deactivated (relative to the corresponding acceptor with a free hydroxy group). Obviously, by using different LGs, further levels of reactivity can be added to increase the synthetic repertoire. However, the essential test for the whole concept, proving its versatility, is its application in oligosaccharide synthesis.

### 6.4.1 Application in oligosaccharide synthesis

In the following schemes (Schemes 6.9 and 6.10) the synthesis of some challenging oligosaccharide targets are discussed, with a focus on generating glycosyl donors and hermaphrodites of differential reactivities.

The first example describes the synthesis of high-mannose-type oligosaccharides, compounds that have attracted particular attention owing to their presence on the envelope glycoprotein gp120 of the human deficiency virus (HIV) [50]. The glycans were selected as possible targets for immunotherapy and for vaccine development [51–55]. Following some preliminary studies [40], the synthesis of a nine-mannose containing fragment bearing a suitable linker for eventual coupling to the protein was



**Scheme 6.9** One-pot strategy for oligosaccharide assembly [41].

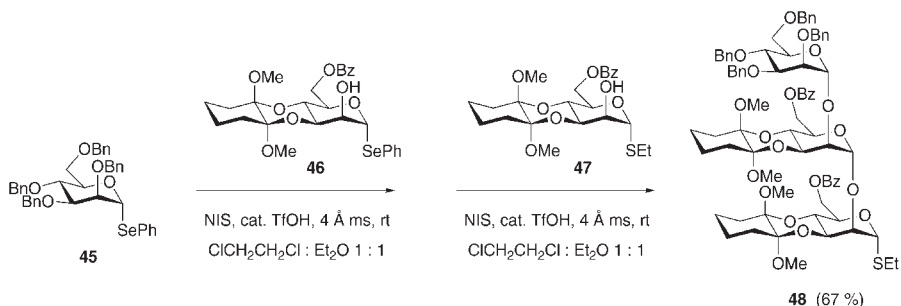
reported [41]. Starting with the highly reactive phenylseleno donors **45** and **46**, the perbenzylated derivative **45** was preferentially activated with NIS/TfOH over the CDA detuned acceptor **46**. The intermediate disaccharide was not isolated, but could be directly coupled with the third component **47** furnishing the key trisaccharide **48** (67%). The second acceptor **47** had the same protecting group pattern as glycoside **46**, but contained an ethylthio LG of low reactivity, hence selective activation afforded the now strongly deactivated trisaccharide **48**. Nevertheless, after the one-pot trisaccharide synthesis, one further glycosidation was achieved: Reaction with



## 6.4.2 Experimental details

### Method 5

Synthesis of (1'*S*,2'*S*)-Ethyl 2-*O*-(2-*O*-(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-mannopyranosyl)-6-*O*-benzoyl-3*O*,4*O*-[1',2'-dimethoxycyclohexan-1',2'-diyl]- $\alpha$ -D-mannopyranosyl)-6-*O*-benzoyl-3*O*,4*O*-[1',2'-dimethoxycyclohexan-1',2'-diyl]-1-thio- $\alpha$ -D-mannopyranoside (**48**) [41].



*Notes and discussion.* Exploiting different reactivity levels, Ley *et al.* succeeded not only in synthesizing the title trisaccharide **48** in high yield, but could also further elaborate the anomeric reactivity to form the corresponding tetrasaccharide **50** and a high-mannose type nonasaccharide [40, 41]. Similar approaches led first to the formation of key intermediates of other oligosaccharides, e.g. a GPI anchor, and finally to the natural product itself [43, 56].

### Materials.

Per- <i>O</i> -benzylated phenylseleno- $\alpha$ -D-mannopyranoside <b>45</b> [8, 39, 41] (357 mg, 0.526 mmol)	assume toxic
(1' <i>S</i> ,2' <i>S</i> )-Phenyl 6- <i>O</i> -benzoyl-3- <i>O</i> ,4- <i>O</i> -[1',2'-dimethoxycyclohexane-1',2'-diyl]-1-seleno- $\alpha$ -D-mannopyranoside ( <b>46</b> ) [24] (247 mg, 0.439 mmol)	assume toxic
(1' <i>S</i> ,2' <i>S</i> )-Ethyl 6- <i>O</i> -benzoyl-3- <i>O</i> ,4- <i>O</i> -[1',2'-dimethoxycyclohexane-1',2'-diyl]-1-thio- $\alpha$ -D-mannopyranoside ( <b>47</b> ) [24] (289 mg, 0.618 mmol)	assume toxic
<i>N</i> -Iodosuccinimide (138 mg, 0.614 mmol and 158 mg, 0.702 mmol)	irritant
Trifluoromethanesulfonic acid (2 × 30 $\mu$ l of a 3% stock solution in 1,2-dichloroethane)	causes severe burns
4 Å molecular sieves (1 g preactivated powder)	
Dry 1,2-dichloroethane (11 ml)	toxic, irritant

Dry diethyl ether (9 ml; distilled from sodium benzophenone ketyl)	extremely flammable, harmful
Celite <sup>®</sup>	
10% sodium thiosulfate solution (20 ml)	irritant
Dichloromethane (170 ml)	toxic, irritant
Sodium sulfate	irritant

#### Equipment.

Round bottomed flasks (10, 25, and 50 ml) with stirrer bars and septa (pressure balance!)  
 Magnetic stirrer  
 Syringes  
 Measuring cylinder  
 Funnel  
 Separating funnel  
 Conical flasks (250 ml)  
 Round bottomed flask (250 ml)  
 Rotary evaporator  
 Flash column chromatography equipment

*Special precautions.* The reaction is carried out under argon in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place a mixture of per-*O*-benzylated phenylseleno- $\alpha$ -D-mannopyranoside **45** [8, 39, 41] (357 mg, 0.526 mmol, 1.2 equiv.), CDA-protected mannose derivative **46** [24] (247 mg, 0.439 mmol, 1.0 equiv.) and powdered 4 Å molecular sieves (800 mg) into a predried flask which is repeatedly evacuated and flushed with argon. After addition of diethyl ether (3 ml) and 1,2-dichloroethane (DCE; 3 ml), stir the suspension for 2.5 h at rt. Cannulate NIS (138 mg, 0.614 mmol, 1.4 equiv.) in diethyl ether (2 ml) and DCE (3 ml) containing trifluoromethanesulfonic acid (TfOH; 30  $\mu$ l of a 3% stock solution of TfOH in DCE) into the mixture of the two sugars whereupon a purple colour appears. After 10 min add a suspension of **47** [24] (289 mg, 0.618 mmol, 1.4 equiv.) and 4 Å molecular sieves (400 mg) in diethyl ether (2 ml) and DCE (2 ml), followed by a second batch of NIS (158 mg, 0.702 mmol, 1.6 equiv.) and TfOH (30  $\mu$ l of stock solution) in diethyl ether (2 ml) and DCE (3 ml). Within 1 h the reaction is complete and no disaccharide can be detected by tlc. Filter the suspension through a pad of celite into a 10% sodium thiosulfate solution (20 ml), add CH<sub>2</sub>Cl<sub>2</sub> (50 ml), separate the layers, and extract the aqueous phase repeatedly with CH<sub>2</sub>Cl<sub>2</sub> (4  $\times$  30 ml). Dry the combined organic layers with sodium sulfate, filter and remove the solvent *in vacuo* to obtain the crude material. Flash column chromatography (hexane/ether, 3:1 to 1:1) furnishes **48** (408 mg, 0.291 mmol,

67%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +125 (*c* 0.76 in CHCl<sub>3</sub>), <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) 8.05 (1H, t, *J* = 7.5 Hz), 7.57 (1H, t, *J* = 7.4 Hz), 7.52–7.19 (23H, m), 7.14–7.09 (2H, m)] (Ar[H]), 5.65 (1H, br s, 1-H''), 5.33 (1H, br s, 1-H), 5.29 (1H, br s, 1-H'), 4.88 (1H, d, *J* = 11.0 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[4]), 4.83 (1H, d, *J* = 12.7 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[2]), 4.74 (1H, d, *J* = 12.2 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[6]), 4.66 (1H, d, *J* = 12.7 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[2]), 4.62 (1H, dd, *J* = 13.8 Hz, 4.2, 6-H<sub>b</sub>), 4.55 (1H, dd, *J* = 11.8 Hz, 1.5, 6-H<sub>b</sub>), 4.51 (1H, d, *J* = 11.5 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[3]), 4.50 (1H, d, *J* = 11.0 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[4]), 4.46 (1H, d, *J* = 11.5 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[3]), 4.44 (1H, d, *J* = 12.2 Hz, PhCH<sub>a</sub>H<sub>b</sub>-[6]), 4.44–4.40 (3H, m, 3-H, 6-H<sub>a</sub>' and 6-H<sub>a</sub>), 4.36 (1H, t, *J* = 9.5 Hz, 4-H), 4.29 (1H, dd, *J* = 9.8 Hz, 2.1, 3-H'), 4.26 (1H, t, *J* = 9.8 Hz, 4-H'), 4.23 (1H, t, *J* = 9.8 Hz, 4-H''), 4.20 (1H, br d, *J* = 2.1 Hz, 2-H'), 4.17 (1H, br, 2-H), 4.17–4.12 (2H, m, 5-H' and 5-H), 4.00 (1H, br, 2-H''), 3.96 (1H, dd, *J* = 9.5, 3.1 Hz, 3-H''), 3.83 (1H, dd, *J* = 10.6, 2.5 Hz, 6-H<sub>b</sub>''), 3.75 (1H, br d, *J* = 9.8 Hz, 5-H''), 3.68 (1H, br d, *J* = 10.6 Hz, 6-H<sub>a</sub>''), [3.16, 3.12, 3.09, 3.08] (4 × 3H, 4 × s, 4 × OMe), 2.61 (2H, m<sub>c</sub>, SCH<sub>2</sub>), 1.80–1.21 (16H, m, 8 × CH<sub>2</sub>-CDA), 1.25 (3H, t, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>).

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# Modern Glycosidation Methods: Orthogonal Glycosidation

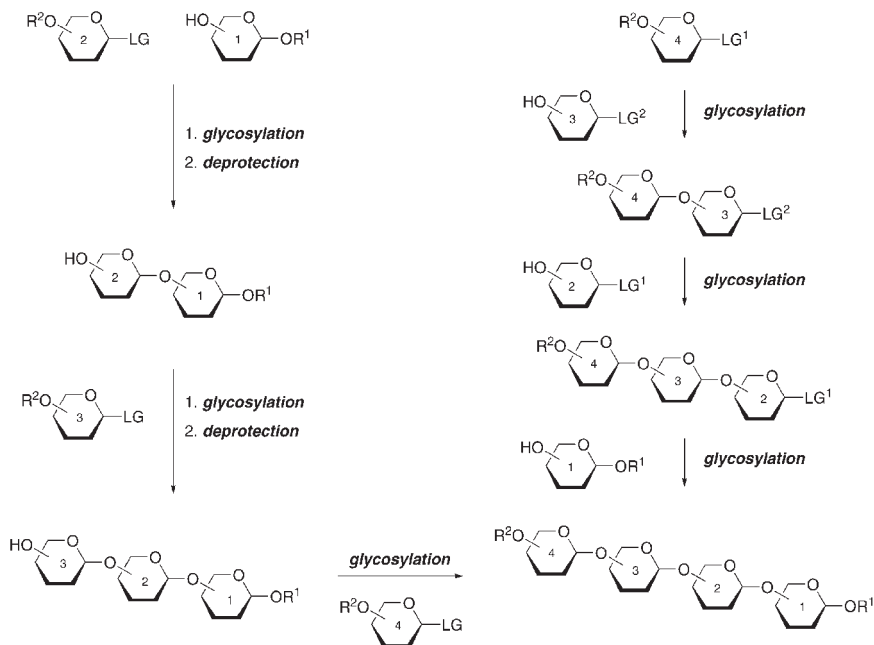
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## 7.1 INTRODUCTION

Although the fundamental role of carbohydrates and glycoconjugates in biological systems has been recognized, access to these molecules remains difficult. The regio- and stereoselective formation of glycosidic bonds is a central problem that is particularly difficult if attempts are made to minimize protecting group manipulations. In a traditional approach to (linear) oligosaccharides, synthesis commences from the reducing end (Scheme 7.1) of the carbohydrate. Initially, a selectively deprotected acceptor (1) is required for reaction with a fully protected glycosyl donor (2) to afford a fully protected disaccharide. Purification and selective deprotection is essential, before the next glycosidation reaction with a fully protected donor (3) can be performed. Following this strategy, synthesis of a tetrasaccharide would require a minimum of five synthetic steps, assuming no additional protecting group manipulations are necessary. Such processes are therefore lengthy and often inefficient. Two approaches have been suggested to minimize the number of synthetic steps required in oligosaccharide synthesis. In both cases oligosaccharide assembly commences from the non-reducing end: the ‘Tuning of Reactivity’ of glycosyl donors (see Chapter 6) allows a consecutive glycosylation–glycosylation–glycosylation sequence to be followed by using a one promotor system, rendering any protecting group manipulations irrelevant. In this way, complex oligosaccharides have been prepared in one-pot strategies. ‘Orthogonal Glycosidation’ approaches offer an alternative approach, and are the subject of this chapter. In this approach a range of glycosyl donors that bear different leaving groups ( $LG^1/LG^2$ ), and that can be selectively activated in the presence of



**Scheme 7.1** Oligosaccharide assembly: traditional concept (left) and by an orthogonal strategy (right).

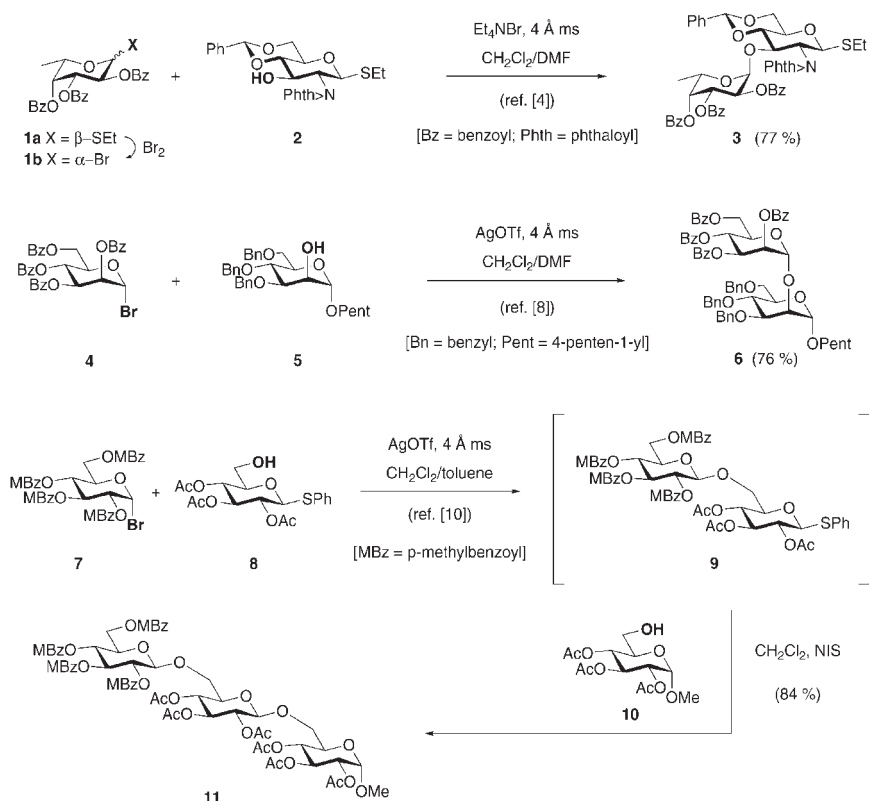
each other, are utilized. Ideally, using this strategy, only three consecutive glycosidations would be needed to synthesize the depicted tetrasaccharide (Scheme 7.1). The correct choice of leaving group at the anomeric position is crucial for the success of this strategy. The aim of this chapter is to describe the different successful combinations of glycosyl donors that can be used for ‘Orthogonal Glycosidations’.

## 7.2 ORTHOGONAL GLYCOSIDATIONS

A highly reactive donor is required for the first glycosidation reaction in the orthogonal glycosidation sequence. However, the acceptor and second donor must be more robust so that no reaction occurs between these latter units upon addition of the first promotor. Thioglycosides [1], phenyl seleno- [2] and 4-penten-1-yl [3] glycosides have all proved to be versatile acceptors for the first glycosidation step. These glycosides are stable under conditions typically used for the activation of the initial donors, which are often glycosyl halides, acetimidates, or sulfoxides. These latter molecules can, however, be activated once the first glycosidation reaction is complete by, for example, addition of iodonium ions.

## 7.2.1 Use of glycosyl bromides

In 1985, Lönn reported a convergent block synthesis of a fucosylated complex-type *N*-glycan nonasaccharide using thioglycosides, e.g. **1a** and **2** (Scheme 7.2), as donors [4, 5]. The preliminary step of this strategy involved the fucosylation of glucosamine **2** with glycosyl bromide **1b**, formed *in situ* by treatment of **1a** with bromine [6]. Disaccharide **3** was formed in good yield (77%). Moreover, after further manipulations the thioglycoside **3** was used as the glycosyl donor itself. Similarly, Sinaÿ *et al.* exploited a glycosyl bromide as the initial glycosyl donor in an orthogonal glycosidation sequence, however, instead of activating this with tetraethylammonium bromide a more common activator, silver trifluoromethanesulfonate (AgOTf), was employed [7]. The reliability of this promotor system was also proven by Fraser-Reid *et al.* Thus mannoside **4** was reacted with 4-penten-1-yl mannoside **5** to give disaccharide **6** [8], a building block that was needed for the assembly of

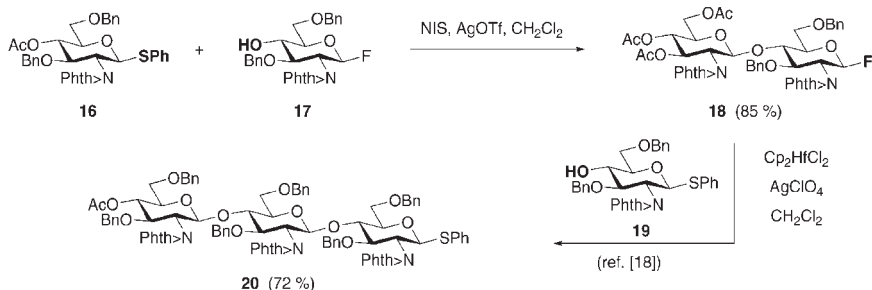
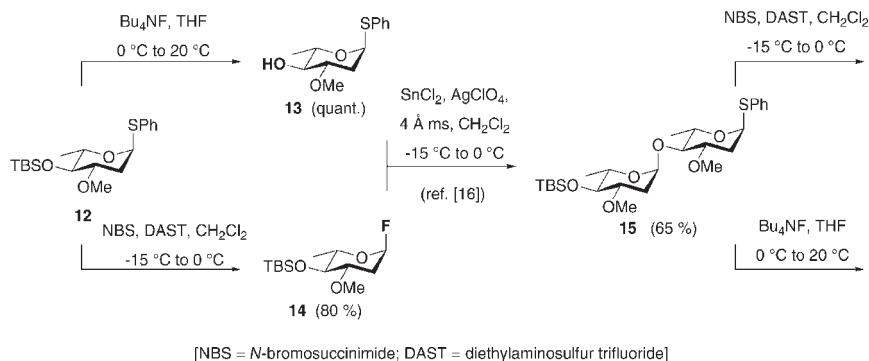


Scheme 7.2 Orthogonal glycosidation: the use of glycosyl bromides.

the pentasaccharide core of the protein membrane anchor found in *Trypanosoma brucei* [9]. Takahashi *et al.* further improved the efficiency of this approach and were the first to employ glycosyl bromides in one-pot sequential glycosidations to form linear and branched oligosaccharides [10–12]. An early example is shown in Scheme 7.2: [10] AgOTf promoted reaction of bromide **7** in the presence of phenyl thioglycoside **8** furnished disaccharide **9** that was not isolated, but further activated by the addition of the thiophilic *N*-iodosuccinimide (NIS) promotor. Reaction with the acceptor **10** gave trisaccharide **11** in high yield. High  $\beta$ -selectivity was observed for both newly formed stereogenic centers due to neighboring group participation.

### 7.2.2 Use of glycosyl fluorides

Glycosyl bromides are usually highly reactive donors and are sometimes difficult to store. Consequently, they are regularly formed *in situ* immediately prior to activation. Glycosyl fluorides are alternative more stable glycosyl donors [13]. They were first utilized for effective glycosidations by Mukaiyama *et al.* in 1981 [14]. Due to their enhanced stability (e.g. on silica), ease of handling, and ability to often afford highly stereoselective reactions, compared with other glycosyl halides, glycosyl fluorides have drawn considerable attention during the last decade. Nicolaou's laboratory was one of the first to make extensive use of glycosyl fluorides (and sulfides). The group developed a protocol to convert thioglycosides to the corresponding glycosyl fluorides, prior to the invention of efficient (thiophilic) thioglycoside promoters [15]. This allowed an iterative block synthesis of a variety of oligosaccharides by a so-called two-stage activation strategy [16, 17]. The usefulness of the method was first demonstrated in the partial synthesis of avermectin B<sub>1a</sub> [16]: starting from oleandrose derivative **12** (Scheme 7.3), the thioglycoside was converted to the hydroxy component **13** (glycosyl acceptor) using tetrabutylammonium fluoride (quant.) and to glycosyl fluoride **14** (glycosyl donor) by treatment with NBS/DAST (80%). Coupling of the two units with SnCl<sub>2</sub>/AgClO<sub>4</sub> yielded disaccharide **15** in 65% yield. Further activation of the anomeric center as the fluoride followed by coupling to the aglycon furnished avermectin B<sub>1a</sub> [16]. Alternatively, selective deprotection of disaccharide **15** would yield a corresponding new acceptor. A decade later Ogawa *et al.* proposed an orthogonal glycosidation strategy that required an efficient activator for thioglycosides: NIS/AgOTf selectively reacted with phenyl thioglycoside **16** in the presence of the orthogonal acceptor **17**. The disaccharide **18** (85%) could subsequently be activated without the need for further manipulations of the newly formed oligosaccharide: upon activation with hafnocene dichloride (Cp<sub>2</sub>HfCl<sub>2</sub>)/AgClO<sub>4</sub> the fluoride **18** coupled to sulfide **19**, yielding trisaccharide **20** in 72% yield [18]. More recent applications of this convergent approach include the synthesis of the tumor-associated antigen Globo-H [19] and the GPI anchor of yeast (*Saccharomyces cerevisiae*) [20]. An extension of the strategy is the one-pot sequential glycosidation, involving initial activation of a fluoride, as reported by Takahashi *et al.* [10].

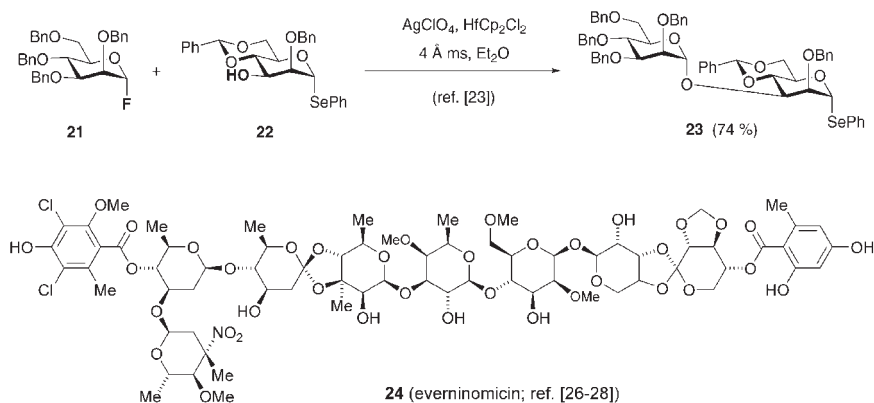


**Scheme 7.3** Orthogonal glycosidation: the use of glycosyl fluorides.

In view of the high utility of phenyl selenoglycosides, especially for glycosylation strategies involving reactivity tuning of glycosyl donors (see Chapter 6), the synthesis of phenyl selenoglycoside acceptors would be advantageous. Thus Ley *et al.* demonstrated that glycosyl fluorides (e.g. **21**) could be readily activated in the presence of a selenide acceptor (e.g. **22**) (Scheme 7.4) [20–23]. Further coupling of the product selenoglycoside saccharide **23** has also been demonstrated. Applications include the synthesis of key intermediates of high-mannose type neoglycolipids (*N*-glycans of gp63, the major surface glycoprotein from *Leishmania mexicana amazonensis*) [23, 24] or dendritic glycoclusters [25]. Nicolaou *et al.* also exploited the combination of glycosyl fluorides and selenides in their synthesis of everninomicin **24** in 1999. [26–28] It is interesting to note that Mehta and Pinto also activated selenides in the presence of sulfides using  $\text{AgOTf}$ , a promotor that is known not to react with thioglycosides [29, 30].

### 7.2.3 Primary activation by Lewis acids

Thioglycosides and 4-pent-1-yl glycosides are inert to activation with Lewis acids. Consequently, all donors that can be activated by treatment with a suitable Lewis



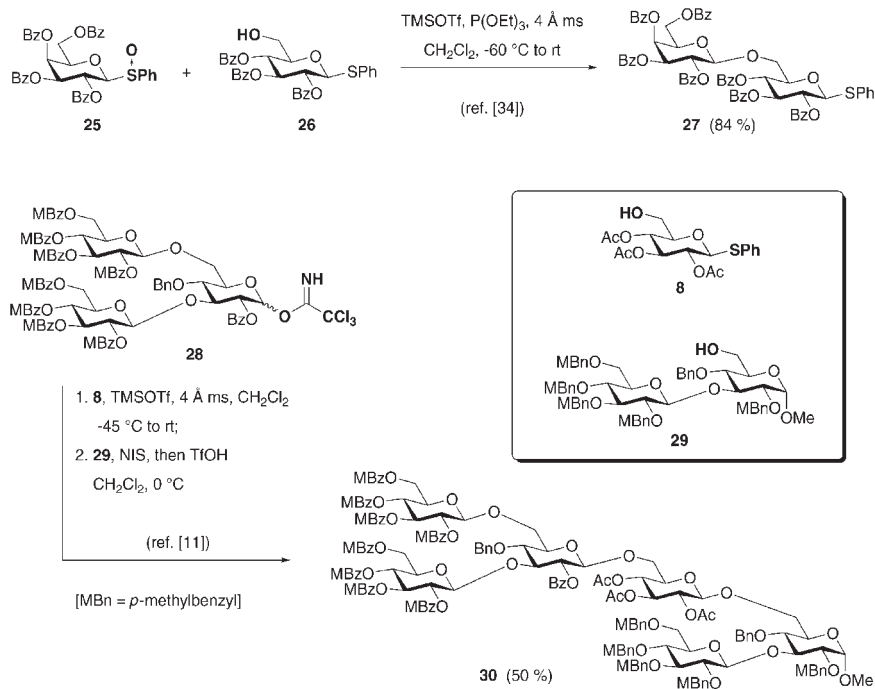
**Scheme 7.4** Orthogonal glycosidation: formation of selenodisaccharide **23**; everninomicin **24**—an application.

acid would be ideal for orthogonal strategies with thioglycosides or 4-penten-1-yl glycosides to assemble oligosaccharides. In 1994, Chenault *et al.* reported a glycosyl transfer reaction using an isoprenyl glycosidic donor and a 4-penten-1-yl acceptor. Primary, selective activation was achieved with trimethylsilyl triflate (TMSOTf); the intermediate disaccharide then underwent a second, successive coupling in one-pot to give a trisaccharide [31].

Thioglycosides have been used more regularly in these strategies. For example, they have been used as latent donors in combination with highly reactive sulfoxides. While Kahne *et al.* first used triflic anhydride [32] and later (in a one-pot two-step synthesis) triflic acid in the presence of methyl propiolate (as a scavenger) [33] to activate the sulfoxide donors, van Boom *et al.* employed TMSOTf/triethylphosphite [34]. Under these reaction conditions, phenylsulfonyl glycoside **25** was readily activated and coupled with thioglycoside **26** yielding disaccharide **27** (Scheme 7.5). Trichloroacetimidates are an alternative orthogonal donor to sulfoxides for use with thioglycoside latent donors.  $\text{BF}_3 \cdot \text{OEt}_2$  [10] or TMSOTf [7, 11] have been used as Lewis acids for their activation in the presence of the thioglycoside. For example, Takahashi *et al.* have elegantly incorporated imidates within one-pot oligosaccharide synthesis: [11] donor **28** reacted selectively with phenyl thioglycoside **8** giving a thioglycoside tetrasaccharide that was not isolated, but further activated with NIS/TfOH. The disaccharide **29** was utilized as the acceptor to afford the hexasaccharide **30**.

## 7.2.4 Summary

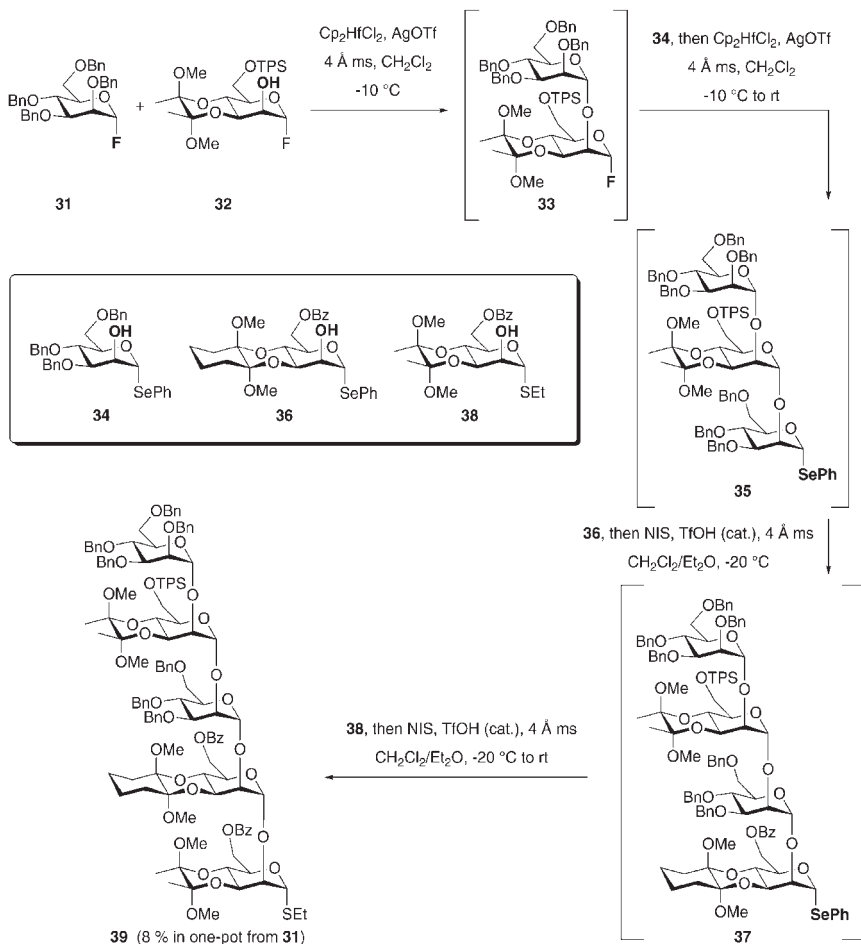
In summary, sulfides, selenides, or 4-penten-1-yl glycosides in combination with halides, trichloroacetates or sulfoxides, have been shown to be ideal building blocks



**Scheme 7.5** Orthogonal glycosidation: primary activation by a Lewis acid.

for orthogonal glycosidation strategies. One-pot sequences have allowed the highly efficient assembly of oligosaccharides, without any tedious protecting group manipulations. However, until recently the number of components that could be combined in this kind of sequential glycosidations was limited to three. In order to further improve the efficiency of oligosaccharide synthesis, a combination of strategies has been developed. In 1997, Ley *et al.* reported the first successful example using the principles of both orthogonal activation and reactivity tuning (See Chapter 6) [20–25]. Five different levels of reactivity were obtained, allowing one-pot synthesis of tetra- and even pentasaccharides from monomeric building blocks [21]. In addition, branched penta- and heptasaccharides could be synthesized in a similar fashion from up to five components [20–22]. An example of this methodology is provided in Scheme 7.6 [20, 22]: starting with the two glycosyl fluorides **31** and **32** (**32** is less reactive due to torsional effects; see Chapter 6), disaccharide **33** was formed upon activation with  $\text{Cp}_2\text{HfCl}_2/\text{AgOTf}$ . After addition of the orthogonal acceptor **34** and a second portion of the promotor, selenide **35** could be obtained after warming the reaction mixture to room temperature. Alternatively, selenide **36** (again: less reactive than saccharide **35** due to torsional effects of the protecting group) and NIS/TfOH as promotor were added giving tetrasaccharide **37**. A final glycosidation was possible,





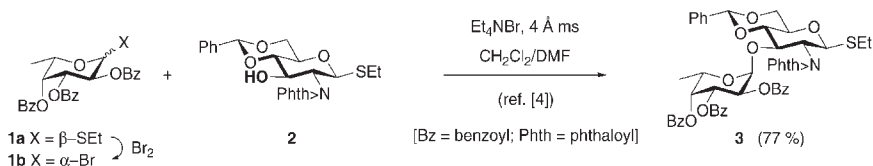
**Scheme 7.6** One-pot strategy for oligosaccharide assembly [20].

when sulfide **38**, an acceptor that for electronic reasons is less reactive than the corresponding selenide **37**, and another portion of the promotor system, were added. Pentasaccharide **39** was obtained in a respectable 8% yield (53% per glycosidation) from monosaccharide **31**. It is interesting to note that target molecule **39** is still a glycosyl donor providing the potential for yet another activation and coupling step. If a glycosyl fluoride were used as the new acceptor, a highly reactive glycoside would be formed, which in principle would be ready for a new one-pot sequence. However, it is not essential to always perform reactions in one-pot: for the rapid and efficient synthesis of oligosaccharides it is only important to note that a combination of strategies will often be most rewarding [20, 23–25].

### 7.2.5 Experimental details

#### Method 1

Synthesis of 3-*O*-(2,3,4-tri-*O*-benzyl- $\alpha$ -L-fucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside (**3**) [4].



*Notes and discussion.* Stable thioglycosides [1] are convenient precursors to more reactive glycosyl donors, e.g. glycosyl bromides [6]. Lönn used this approach to synthesize and selectively activate a halide in the presence of a second, masked donor [4]. The disaccharide thus obtained could be further reacted with glycosyl acceptors.

#### Materials.

Ethyl 2,3,4-tri- <i>O</i> -benzyl-1-thio- $\beta$ -L-fucopyranoside ( <b><math>\beta</math>-1a</b> ) [4]	assume toxic
(1.52 g, 2.92 mmol)	
Bromine (165 $\mu$ l, 3.20 mmol)	toxic, corrosive
Dry dichloromethane (20 ml; distilled from calcium hydride)	toxic, irritant
Toluene (75 ml)	irritant, flammable
Dry dichloromethane (3 ml; distilled from calcium hydride)	toxic, irritant
Ethyl 4,6-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside ( <b>2</b> ) [4] (0.75 g, 1.70 mmol)	assume toxic
4 Å molecular sieves (3 g preactivated powder)	
Tetraethylammonium bromide (0.67 g, 3.19 mmol)	
<i>N,N</i> -Dimethylformamide (4 ml; distilled from molecular sieves 4 Å)	irritant
Ethanol (1 ml)	irritant, highly flammable
Celite <sup>®</sup>	
Toluene (50 ml)	irritant, flammable
10% aqueous sodium bicarbonate solution	

*Equipment.*

Two round bottomed flask (50 ml) with stirrer bar and septum (pressure balance!)  
 Magnetic stirrer  
 Silver foil  
 Syringes  
 Measuring cylinder  
 Funnel  
 Separating funnel  
 Conical flasks (100 and 250 ml)  
 Round bottomed flask (100 ml)  
 Rotary evaporator  
 Flash column chromatography equipment

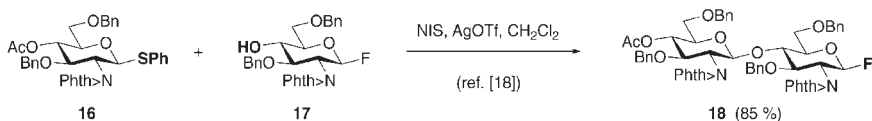
*Special precautions.* The reaction is carried out under argon in predried glassware; the reaction vessel should be wrapped in silver foil. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place a solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio- $\beta$ -L-fucopyranoside ( $\beta$ -**1a**) [4] (1.52 g, 2.92 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 ml) in a predried round bottomed flask. Add bromine (165  $\mu\text{l}$ , 3.20 mmol) carefully by syringe at 0 °C. After 20 min, concentrate the solution and co-distil with toluene ( $3 \times 25$  ml). Add  $\text{CH}_2\text{Cl}_2$  (3 ml) and syringe the solution into a stirred mixture of ethyl 4,6-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside (**2**) [4] (0.75 g, 1.70 mmol), tetraethylammonium bromide (0.67 g, 3.19 mmol), and 4 Å molecular sieves (3 g preactivated powder) in *N,N*-dimethylformamide (4 ml). After 18 h, add ethanol (1 ml) and continue to stir for 1 h. Filter the mixture through a pad of Celite<sup>®</sup>, dilute with toluene, wash with aqueous sodium bicarbonate solution and water. Separate the organic layer and concentrate under reduced pressure. The product **3** is obtained after chromatographic purification (eluent: toluene–ethyl acetate 15:1), yielding 1.18 g (1.31 mmol, 77%) as a syrup;  $[\alpha]_{\text{D}}^{25} = -36$  (*c* 1.0 in  $\text{CH}_2\text{Cl}_2$ );  $^{13}\text{C}$  NMR (25 MHz,  $\text{CDCl}_3$ ): 14.0 ( $\text{CH}_3\text{CH}_2$ ), 15.5 (C-6'), 23.1 ( $\text{CH}_3\text{CH}_2$ ), 53.7 (C-2), 66.4, 67.7, 69.7, 71.8, 72.2, 73.8, 74.6, 75.5, 77.1, 78.7, 80.9, 81.1 (C-1, C-3, C-4, C-5, C-2', C-3', C-4', C-5', and  $3\text{PhCH}_2$ ), 98.5 (C-1',  $J = 167$  Hz), 100.2 (PhCH), 122.3, 125.1, 126.1–128.0, 131.0, 132.9, 136.2, 137.4, 137.6, 137.9 (Ar-C), 166.8, 167.3 (Phth).

*Method 2*

Synthesis of 4-*O*-(4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl fluoride (**18**) [18].



*Notes and discussion.* Glycosyl fluorides have proven excellent donors in oligosaccharide synthesis [13, 35]. Nicolaou *et al.* were the first to use them efficiently in a two-stage glycosidation procedure [15, 16]. This strategy has been exploited in the preparation of *Rhynchosporides* [36]. The orthogonal strategy by Ogawa *et al.* further reduced the need for subsequent manipulation of the oligosaccharide: activation of either the glycosyl fluoride or the thioglycoside was shown to be possible [18].

*Materials.*

Phenyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside (**16**) [18] (266 mg, 0.43 mmol)      assume toxic

3,6-Di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl fluoride (**17**) [18] (314 mg, 0.64 mmol)      assume toxic

Silver trifluoromethanesulfonate (10.6 mg, 0.04 mmol; dried azeotropically with toluene and *in vacuo* before use)      irritant

*N*-Iodosuccinimide (126 mg, 0.56 mmol)      irritant

4 Å molecular sieves (1 g preactivated powder)

Dry dichloromethane (5 ml; distilled from calcium hydride)      toxic, irritant

Celite<sup>®</sup>

Ethyl acetate (20 ml)      highly flammable, irritant

Saturated sodium bicarbonate solution (20 ml)

Magnesium sulfate

*Equipment.*

Round bottomed flask (50 ml) with stirrer bar and septa (pressure balance!)

Magnetic stirrer

Cooling bath with thermometer

Measuring cylinder

Funnel

Separating funnel

Conical flasks (100 ml)

Round bottomed flask (100 ml)

Rotary evaporator

Column chromatography equipment

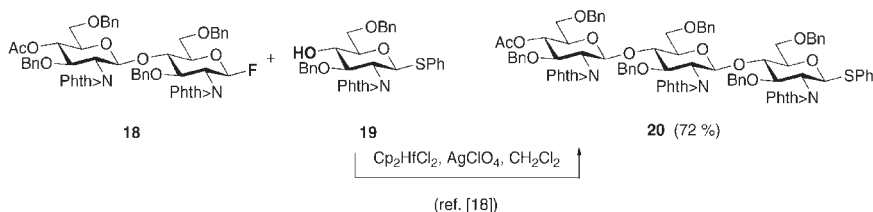
*Special precautions.* The reaction is carried out under argon in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place phenyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside (**16**) [18] (266 mg, 0.43 mmol), 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl fluoride (**17**) [18] (314 mg, 0.64 mmol), and 4 Å molecular sieves (1 g preactivated powder) into a predried round bottomed flask. Add silver trifluoromethanesulfonate (10.6 mg, 0.04 mmol; dried azeotropically with toluene and *in vacuo* before use) and *N*-iodosuccinimide (126 mg, 0.56 mmol) at  $-50^{\circ}\text{C}$ . Stir the mixture for 6 h while the cooling bath temperature is allowed to become  $20^{\circ}\text{C}$ . Dilute the reaction mixture with ethyl acetate (20 ml) and add saturated aqueous sodium bicarbonate solution (20 ml). After stirring for 5 min, filter the mixture through a pad of Celite<sup>®</sup>. Separate the organic layer, wash with water, dry ( $\text{MgSO}_4$ ), filter and concentrate *in vacuo* to obtain an oil. Purification by column chromatography (bio-beads S-X1; eluent: toluene–ethyl acetate 3:1) furnishes the disaccharide **18** (362 mg, 0.36 mmol, 85%);  $[\alpha]_{\text{D}}^{25} = +40.9$  (*c* 1.20 in  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 7.90–6.75 (33H, m, Ar-H), 5.69 (1H, d,  $J = 53.8$  Hz, 1-H), 5.34 (1H, d,  $J = 8.3$  Hz, 1'-H), 5.16 (1H, t,  $J = 9.4$  Hz, 4'-H), 4.28 (1H, dd,  $J = 10.6, 9.4$  Hz, 2'-H), 1.93 (3H, s,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (90 MHz,  $\text{CDCl}_3$ ): 20.9 ( $\text{CH}_3$ ), 55.5 ( $J = 21.9$  Hz, C-2), 56.1 (C-2'), 67.7, 69.3, 72.6, 72.9, 73.4, 73.5, 73.9, 74.4, 74.5 ( $J = 4.9$  Hz, C-5), 75.4, 75.5 ( $J = 7.3$  Hz, C-3), 76.8, 96.9 (C-1'), 104.7 ( $J = 215.9$  Hz, C-1).

### Method 3

Synthesis of phenyl 4-*O*-[4-*O*-(4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside (**20**) [18].



**Notes and discussion.** This orthogonal strategy described by Ogawa *et al.* exploits the fact that thioglycosides can be activated without affecting glycosyl fluorides and *vice versa* [18]. The procedure can be conveniently repeated to give larger oligosaccharides.

### Materials.

4-*O*-(4-*O*-Acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl fluoride (**18**) [18] (325 mg, 0.32 mmol) assume toxic

Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside (**19**) [18] (282 mg, 0.49 mmol) assume toxic

Hafnocene dichloride (160 mg, 0.42 mmol)	irritant
Silver perchlorate (174 mg, 0.84 mmol)	corrosive, toxic
4 Å molecular sieves (1 g preactivated powder)	
Dry dichloromethane (5 ml; distilled from calcium hydride)	toxic, irritant
Celite®	
Ethyl acetate (20 ml)	highly flammable, irritant
Saturated sodium bicarbonate solution (20 ml)	
Magnesium sulfate	

### Equipment.

Round bottomed flask (50 ml) with stirrer bar and septa (pressure balance!)  
 Magnetic stirrer  
 Cooling bath with thermometer  
 Measuring cylinder  
 Funnel  
 Separating funnel  
 Conical flasks (100 ml)  
 Round bottomed flask (100 ml)  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* The reaction is carried out under argon in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

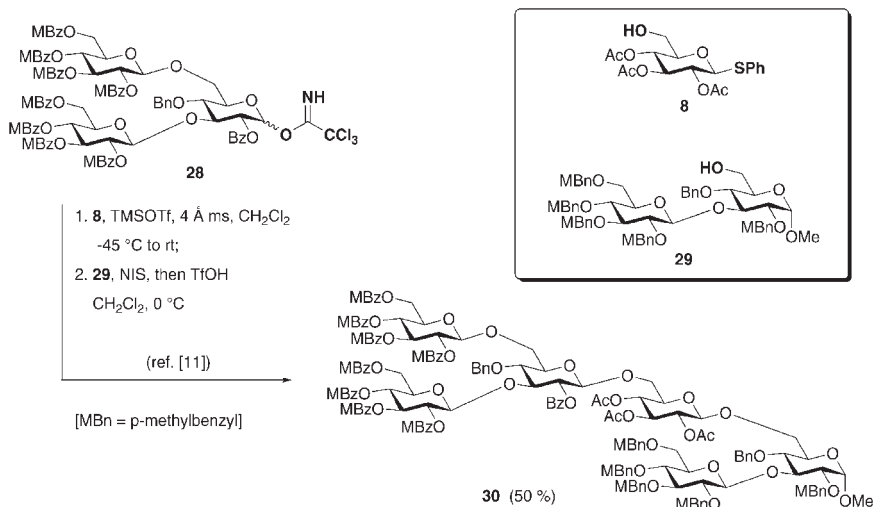
The operator must wear safety glasses, gloves and a laboratory coat.

Place 4-*O*-(4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl fluoride (**18**) [18] (325 mg, 0.32 mmol), phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**19**) [18] (282 mg, 0.49 mmol) and 4 Å molecular sieves (1 g preactivated powder) into a predried round bottomed flask. Add hafnocene dichloride (160 mg, 0.42 mmol) and silver perchlorate (174 mg, 0.84 mmol) at -78 °C. Stir the mixture for 4 h while the cooling bath temperature is allowed to become 20 °C. Dilute the reaction mixture with ethyl acetate (20 ml) and add saturated aqueous sodium bicarbonate solution (20 ml). After stirring for 5 min, filter the mixture through a pad of Celite®. Separate the organic layer, wash with water, dry (MgSO<sub>4</sub>), filter and concentrate *in vacuo* to obtain an oil. Purification by column chromatography (bio-beads S-X1; eluent: toluene-ethyl acetate 3:1) furnishes the trisaccharide **20** (367 mg, 0.23 mmol, 72%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +54.4 (*c* 0.95 in CHCl<sub>3</sub>); <sup>13</sup>C NMR (90 MHz, CDCl<sub>3</sub>): 20.9 (CH<sub>3</sub>), 54.7, 56.2, 56.5 (C-2, C-2', C-2''),

66.9, 68.2, 69.4, 72.3, 72.5, 72.8, 73.1, 73.5, 73.9, 74.3, 74.6, 75.3, 76.0, 76.7, 76.8, 77.5, 78.7, 83.3 (C-1), 96.7, 97.0 (C-1', C-1'').

#### Method 4

Synthesis of methyl 6-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-{4-*O*-benzyl-2-*O*-benzoyl-3,6-di-*O*-[2,3,4,6-tetra-*O*-(4-methylbenzoyl)- $\beta$ -D-glucopyranosyl]- $\beta$ -D-glucopyranosyl]- $\beta$ -D-glucopyranosyl]-4-*O*-benzyl-2-*O*-(4-methylbenzyl)-3-*O*-[2,3,4,6-tetra-*O*-(4-methylbenzyl)- $\beta$ -D-glucopyranosyl]- $\beta$ -D-glucopyranoside (**30**) [11].



**Notes and discussion.** Trichloroacetimidates are versatile glycosyl donors and have played crucial roles in oligosaccharide assembly strategies [37, 38]. Takahashi *et al.* were the first to apply this strategy to a one-pot, two-step orthogonal glycosidation protocol [11].

#### Materials.

4-*O*-Benzyl-2-*O*-benzoyl-3,6-di-*O*-[2,3,4,6-tetra-*O*-(4-methylbenzoyl)- $\beta$ -D-glucopyranosyl]- $\alpha/\beta$ -D-glucopyranosyl trichloroacetimidate (**28**) [11] (65 mg, 36  $\mu$ mol) assume toxic

Phenyl 2,3,4-tri-*O*-acetyl-1-thio- $\beta$ -D-glucopyranoside (**8**) [11] (14 mg, 36  $\mu$ mol) assume toxic

Methyl 4-*O*-benzyl-2-*O*-(4-methylbenzyl)-3-*O*-[2,3,4,6-tetra-*O*-(4-methylbenzyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside (**29**) [11] (43 mg, 43  $\mu$ mol) assume toxic

Trimethylsilyl trifluoromethanesulfonate (1  $\mu$ l) corrosive

*N*-Iodosuccinimide (83.0 mg, 370  $\mu$ mol) irritant

Saturated trifluoromethanesulfonic acid in dichloromethane (150 $\mu$ mol)	causes severe burns
4 Å molecular sieves (0.5 g preactivated powder)	
Dry dichloromethane (3 ml; distilled from calcium hydride)	toxic, irritant
Triethylamine (1 ml)	corrosive, harmful
Celite <sup>®</sup>	
Dichloromethane (20 ml)	toxic, irritant

### Equipment.

Round bottomed *Schlenk*-flask (25 ml) with stirrer bar and septum (pressure balance!)

Magnetic stirrer

Syringe

Measuring cylinder

Funnel

Separating funnel

Conical flasks (100 ml)

Round bottomed flask (50 ml)

Rotary evaporator

Flash column chromatography equipment

*Special precautions.* The reaction is carried out under nitrogen in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

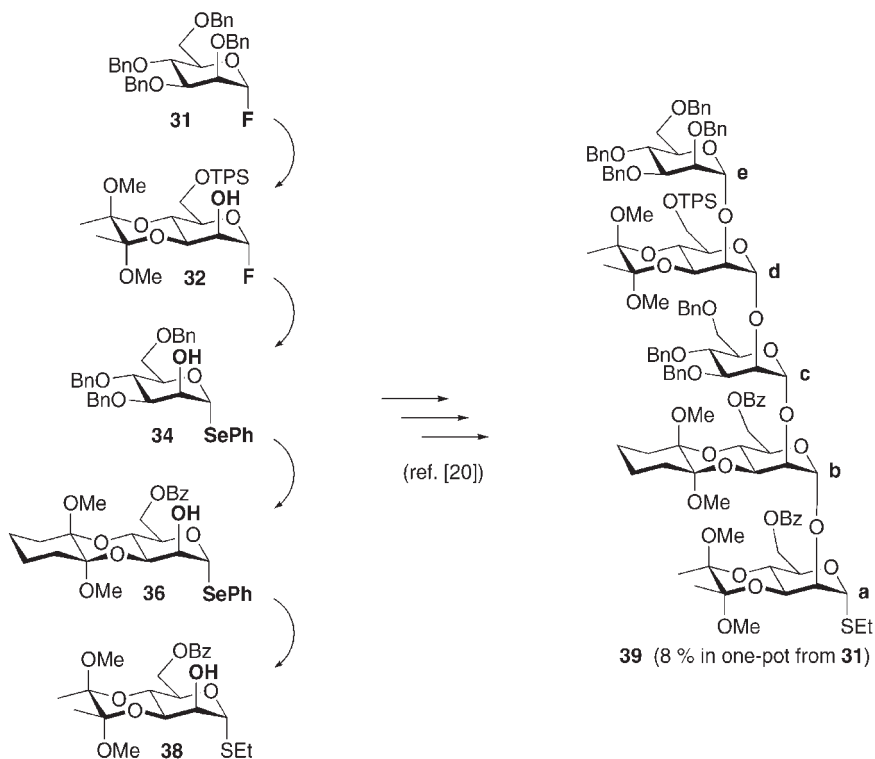
Place 4-*O*-benzyl-2-*O*-benzoyl-3,6-di-*O*-[2,3,4,6-tetra-*O*-(4-methylbenzoyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ / $\beta$ -D-glucopyranosyl trichloroacetimidate (**28**) [11] (65 mg, 36  $\mu$ mol), phenyl 2,3,4-tri-*O*-acetyl-1-thio- $\beta$ -D-glucopyranoside (**8**) [11] (14 mg, 36  $\mu$ mol), and 4 Å molecular sieves (0.5 g preactivated powder) into a predried flask. Add CH<sub>2</sub>Cl<sub>2</sub> (3 ml) and syringe trimethylsilyl trifluoromethanesulfonate (1  $\mu$ l) into the stirring mixture at 45 °C. After 3 min, remove the cooling bath and raise the reaction temperature to the room temperature. At this stage tlc analysis should confirm the completion of the first reaction step. Add methyl 4-*O*-benzyl-2-*O*-(4-methylbenzyl)-3-*O*-[2,3,4,6-tetra-*O*-(4-methylbenzyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside (**29**) [11] (43 mg, 43  $\mu$ mol) and *N*-iodosuccinimide (83 mg, 370  $\mu$ mol) and stir the mixture for another 15 min to remove traces of water. Syringe a saturated solution of trifluoromethanesulfonic acid in CH<sub>2</sub>Cl<sub>2</sub> (~ 150  $\mu$ mol) into the mixture at 0 °C until the red color ceases to be immediately discharged. After tlc analysis indicates completion of the reaction, quench the mixture by adding triethylamine (1 ml) and filter through a pad of Celite<sup>®</sup> flushing with CH<sub>2</sub>Cl<sub>2</sub>. Concentrate the organic layer under reduced pressure and purify the crude product by



column chromatography (eluent: 1% ethyl acetate in toluene) furnishing product **30** (52 mg, 18  $\mu$ mol, 50%);  $[\alpha]_D^{25} = +13.5$  (*c* 1.54 in  $\text{CHCl}_3$ );  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ): 20.5, 20.6, 20.7, 21.0, 21.51, 21.56, 22.5, 22.6, 23.7, 54.9, 63.0, 66.6, 67.4, 68.1, 68.7, 68.9, 69.0, 69.5, 69.8, 71.5, 71.8, 71.9, 72.1, 72.72, 72.79, 72.8, 73.2, 73.3, 74.0, 74.2, 74.4, 74.6, 74.7, 74.8, 75.3, 75.4, 75.7, 75.8, 77.1, 78.0, 80.1, 80.9, 83.3, 84.7 [97.5, 99.6, 99.8, 100.3, 101.1, 102.5 (anomeric C)], 126.0, 126.2, 126.5, 126.7, 126.9, 127.3, 127.4, 127.61, 127.65, 127.8, 127.9, 128.0, 128.12, 128.17, 128.2, 128.4, 128.5, 128.8, 128.9, 129.1, 129.4, 129.6, 129.7, 134.9, 135.2, 135.4, 135.7, 137.01, 137.07, 137.2, 143.5, 143.70, 143.75, 143.8, 143.9 [164.3, 164.9, 165.12, 165.15, 165.57, 165.7, 166.0, 166.1, 168.8, 169.7, 170.1] ( $\text{C}=\text{O}$ ).

### Method 5

Synthesis of (2'*S*,3'*S*,1''*S*,2''*S*,2'''*S*,3'''*S*) ethyl 2-*O*-[2-*O*-{3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-{2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-mannopyranosyl}-6-*O*-*tert*-butyldiphenylsilyl-3*O*,4*O*-(2'''',3'''-dimethoxybutan-2',3'-diyl)- $\alpha$ -D-mannopyranosyl]-6-*O*-benzoyl-3*O*,4*O*-(1',2'-dimethoxycyclohexan-1'',2''-diyl)- $\alpha$ -D-mannopyranosyl]-6-*O*-benzoyl-3*O*,4*O*-(2',3'-dimethoxybutan-2',3'-diyl)-1-thio- $\alpha$ -D-mannopyranoside (**39**) [20].



*Notes and discussion.* The power of modern glycosidation methods was demonstrated by combining an orthogonal (F *versus* SePh) and a reactivity tuning (see Chapter 6) approach. In a one-pot, four step reaction, a pentasaccharide was formed in 8% yield from donor **31** [20]. Further applications in efficient oligosaccharide synthesis have also been reported [20, 21, 23–25].

*Materials.*

2,3,4,6-Tetra-*O*-benzyl- $\alpha$ -D-mannopyranosyl fluoride (**31**)  
[20] (50 mg, 92  $\mu$ mol)

(2'*S*,3'*S*) 6-*O*-*tert*-butyldiphenylsilyl-3*O*,4*O*-(2',3'-dimethoxybutan-2',3'-diyl)- $\alpha$ -D-mannopyranosyl fluoride (**32**)  
[20] (59 mg, 110  $\mu$ mol)

Phenyl 3,4,6-tri-*O*-benzyl-1-seleno- $\alpha$ -D-mannopyranoside (**34**) [23] (64 mg, 110  $\mu$ mol)

(1'*S*,2'*S*) Phenyl 6-*O*-benzoyl-3*O*,4*O*-(1',2'-dimethoxycyclohexan-1'',2''-diyl)-1-seleno- $\alpha$ -D-mannopyranoside (**36**) [39] (68 mg, 120  $\mu$ mol)

(2'*S*,3'*S*) Ethyl 6-*O*-benzoyl-3*O*,4*O*-(2',3'-dimethoxybutan-2',3'-diyl)-1-thio- $\alpha$ -D-mannopyranoside (**38**) [20] (57 mg, 130  $\mu$ mol)

Silver trifluoromethanesulfonate (57 mg, 220  $\mu$ mol and 85 mg, 330  $\mu$ mol; dried azeotropically with toluene and *in vacuo* before use) irritant

Hafnocene dichloride (43 mg, 110  $\mu$ mol and 64 mg, 170  $\mu$ mol) irritant

*N*-Iodosuccinimide (26 mg, 110  $\mu$ mol and 28 mg, 120  $\mu$ mol) irritant

Trifluoromethanesulfonic acid (2  $\times$  50  $\mu$ l of a 3% stock solution in 1,2-dichloromethane–ether 1:1) causes severe burns

4 Å molecular sieves (0.60 g and 0.40 g preactivated powder)

Dry dichloromethane (3  $\times$  0.60 ml) toxic, irritant

Dry diethyl ether (0.60 ml; distilled from sodium benzo-phenone ketyl) extremely flammable, harmful

Celite<sup>®</sup>

10% Sodium thiosulfate solution (20 ml) irritant

Diethyl ether (50 ml) extremely flammable, harmful

Saturated sodium bicarbonate solution (20 ml)

Magnesium sulfate

*Equipment.*

Round bottomed flasks ( $4 \times 10$  ml) with stirrer bars and septa (pressure balance!)

Schlenk flask fitted with a solid addition tube (25 ml)

Magnetic stirrer

Syringes

Measuring cylinder

Funnel

Separating funnel

Conical flasks (250 ml)

Round bottomed flask (250 ml)

Rotary evaporator

Flash column chromatography equipment

*Special precautions.* The reaction is carried out under argon in predried glassware. Molecular sieves must be predried in the oven and activated for 10 min under vacuum at 300 °C.

The operator must wear safety glasses, gloves and a laboratory coat.

Place silver trifluoromethanesulfonate (57 mg, 220  $\mu$ mol), powdered 4 Å molecular sieves (600 mg) and hafnocene dichloride (43 mg, 110  $\mu$ mol) into a predried Schlenk flask (fitted with a solid addition tube containing a second portion of the promotor mixture) which is repeatedly evacuated and flushed with argon. Suspend the first portion of the activator in  $\text{CH}_2\text{Cl}_2$  (0.6 ml) and stir for 10 min before cooling to 10 °C. Add a solution of 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-mannopyranosyl fluoride (**31**) [20] (50 mg, 92  $\mu$ mol) and (2'*S*,3'*S*) 6-*O*-*tert*-butyldiphenylsilyl-3*O*,4*O*-(2',3'-dimethoxybutan-2',3'-diyl)- $\alpha$ -D-mannopyranosyl fluoride (**32**) [20] (59 mg, 110  $\mu$ mol) in  $\text{CH}_2\text{Cl}_2$  (0.6 ml) *via* syringe. Continue to stir at this temperature for 30 min, before adding a solution of phenyl 3,4,6-tri-*O*-benzyl-1-seleno- $\alpha$ -D-mannopyranoside (**34**) [23] (64 mg, 110  $\mu$ mol) in  $\text{CH}_2\text{Cl}_2$  (0.6 ml) *via* syringe. After addition of the second portion of the promotor mixture, silver trifluoromethanesulfonate (85 mg, 330  $\mu$ mol), powdered 4 Å molecular sieves (400 mg) and hafnocene dichloride (64 mg, 170  $\mu$ mol) from the addition tube, warm the mixture to room temperature over 1.5 h. Syringe a solution of (1'*S*,2'*S*) phenyl 6-*O*-benzoyl-3*O*,4*O*-(1',2'-dimethoxycyclohexan-1'',2''-diyl)-1-seleno- $\alpha$ -D-mannopyranoside (**36**) [39] (68 mg, 120  $\mu$ mol) in  $\text{Et}_2\text{O}$  (0.60 ml) into the mixture, before it is cooled to -20 °C. Add NIS (26 mg, 110  $\mu$ mol) in one portion, immediately followed by trifluoromethanesulfonic acid (TfOH; 50  $\mu$ l of a 3% stock solution of TfOH in  $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$  1:1). Continue to stir for a further 30 min, before cannulating a solution of (2'*S*,3'*S*) ethyl 6-*O*-benzoyl-3*O*,4*O*-(2',3'-dimethoxybutan-2',3'-diyl)-1-thio- $\alpha$ -D-mannopyranoside (**38**) [20] (57 mg, 130  $\mu$ mol) in  $\text{CH}_2\text{Cl}_2$  (0.6 ml) into the mixture, immediately followed by trifluoromethanesulfonic acid (TfOH; 50  $\mu$ l

of a 3% stock solution of TfOH in  $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$  1:1). After 1 h at room temperature, filter the suspension through a pad of Celite<sup>®</sup>. Rinse the filter-cake with  $\text{Et}_2\text{O}$  (50 ml), wash the combined filtrates with aqueous sodium bicarbonate solution (20 ml) and dry over magnesium sulfate. Filtration and concentration under reduced pressure provides the crude product as a yellow oil that is purified by flash column chromatography (petroleum ether–ether, 4:1–1:1), followed by size exclusion chromatography on Sephadex LH-20. The product **39** is isolated as a foam, yield: 17 mg (7.3  $\mu\text{mol}$ , 8%);  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ): 5.69 (1H, s,  $1^{\text{e}}\text{-H}$ ), 5.51 (1H, s,  $1^{\text{d}}\text{-H}$ ), 5.33 (1H, s,  $1^{\text{a}}\text{-H}$ ), 5.24 (1H, s,  $1^{\text{c}}\text{-H}$ ), 5.20 (1H, s,  $1^{\text{b}}\text{-H}$ );  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ): 84.1 ( $\text{C-1}^{\text{a}}$ ), 98.2 ( $\text{C-1}^{\text{e}}$ ), 99.7 ( $\text{C-1}^{\text{c}}$ ,  $\text{C-1}^{\text{d}}$ ), 100.0 ( $\text{C-1}^{\text{b}}$ ).

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# The Stereoselective Synthesis of $\beta$ -Mannosides

Seth C. Ennis and Helen M.I. Osborn

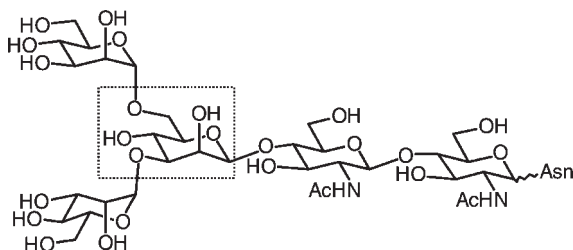
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Reading RG6 6AD, UK*

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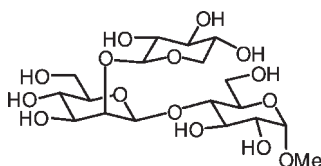
## 8.1 THE NATURAL OCCURRENCE OF $\beta$ -MANNOSIDES

The prevalence of  $\beta$ -mannoside residues in glycoconjugates and other naturally occurring compounds has resulted in the development of a number of methodologies towards their synthesis [1, 2]. The most important class of compounds that contain a  $\beta$ -mannoside linkage are the *N*-linked glycoproteins. A  $\beta$ -mannoside linkage resides within an asparagine bound core pentasaccharide structure, which is common to all *N*-linked glycoproteins (Figure 8.1) [3–5]. These *N*-glycoproteins are classified into four main groups according to the nature of the carbohydrates that substitute the core pentasaccharide structure, namely high mannose, complex, hybrid and poly-*N*-acetylglucosamine [6].

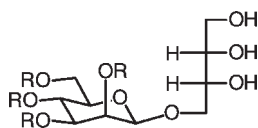
Other sources of naturally occurring compounds containing  $\beta$ -mannoside units include plant polysaccharides [7] and *O*-specific polysaccharides of Gram-negative bacteria [8]. A  $\beta$ -mannoside linkage is also present in the trisaccharide component of a glycosphingolipid found in the fresh water bivalve *Hyriopsis schelegelii*, the synthesis of which will be discussed later in this chapter (Figure 8.2) [9]. Microbial extracellular glycolipids known as MELs are a class of biosurfactants produced by the yeast *Candida antarctica* T-34 [10]. MELs have been shown to induce apoptosis and differentiation in malignant mouse melanoma cells [11], and inhibit the growth of the human promyelocytic leukaemia cell line HL 60 [12]. Structurally, MELs consist of a  $\beta$ -mannoside of *meso*-erythritol and are classified according to the degree of acylation on the mannoside hydroxyl groups (Figure 8.3). A class of complex carbohydrate antibiotics known as the Everninomicins, which are known to be active against drug resistant pathogens such as *Staphylococci* and *Enterococci*, also contain



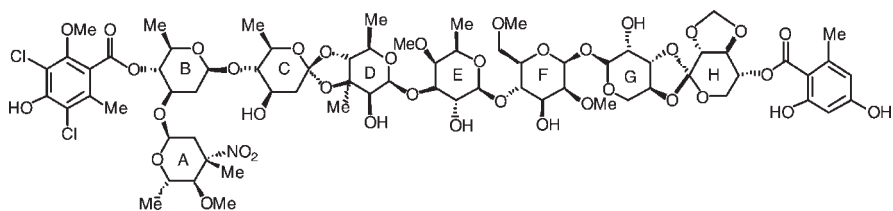
**Figure 8.1** Core pentasaccharide structure of *N*-linked glycoproteins. The dashed box highlights the  $\beta$ -mannoside unit.



**Figure 8.2** The trisaccharide component of the *H. schlegelii* glycosphingolipid.

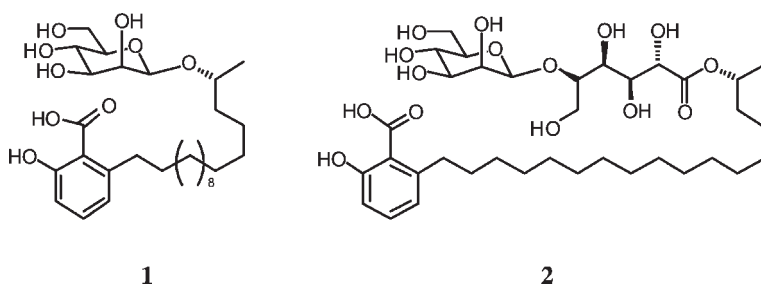


**Figure 8.3** Extracellular glycolipid MEL (R = acyl).



**Figure 8.4** Everninomicin 13,384-1. A  $\beta$ -mannoside linkage connects the FG ring system.

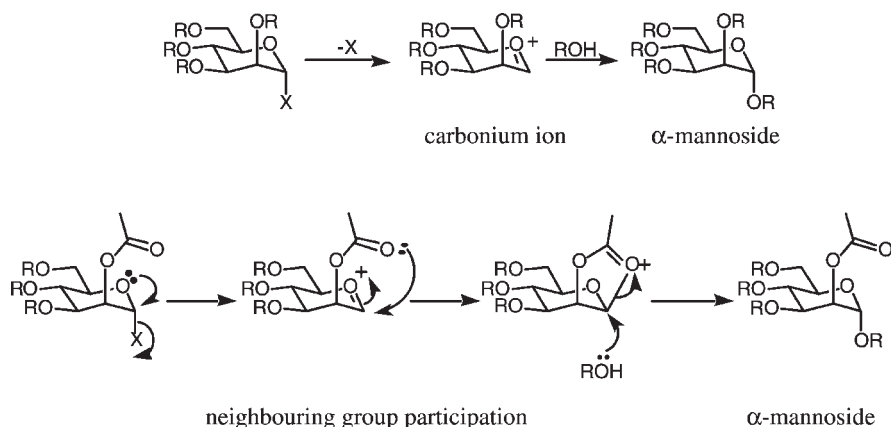
a  $\beta$ -mannoside moiety [13]. The synthesis of the  $\beta$ -mannoside linkage of Everninomicin 13,3841 has been successfully achieved by Nicolaou and co-workers (Figure 8.4) [14]. The fungal metabolite  $\beta$ -mannosyl salicylic acid derivatives **1** and **2**, synthesised by Furstner [15] and Tatsuta [16], respectively, are known to be novel inhibitors of GABA<sub>A</sub>/benzodiazepine chloride receptor ion channels (Figure 8.5). Finally, an *O*-antigen of the *Salmonella* serogroups E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub> and E<sub>4</sub> all contain  $\beta$ -mannoside units as part of a repeating trisaccharide oligomer [17].



**Figure 8.5** Fungal metabolites containing a  $\beta$ -mannoside linkage.

## 8.2 PROBLEMS ASSOCIATED WITH $\beta$ -MANNOSIDE SYNTHESIS

The synthesis of a  $\beta$ -mannoside bond is widely considered to be the most challenging bond formation in carbohydrate chemistry. The problems encountered with the synthesis of a  $\beta$ -mannoside linkage are two-fold. Firstly, electronegative groups bound to the anomeric carbon favour an axial configuration due to the propensity of the anomeric effect. This is caused by the  $n \rightarrow \sigma^*$  interaction of the ring oxygen's lone pair with the anti-bonding orbital of the anomeric carbon [1]. As a consequence, any generation of a carbonium ion favours the formation of an  $\alpha$ -mannoside linkage, both thermodynamically and kinetically, and therefore must be avoided [18]. The use of neighbouring group participation, whereby the mannoside donor possesses a 2-*O*-ester protecting group, is also ineffective as this results in the formation of an  $\alpha$ -mannoside (Figure 8.6) [18]. Thus in order to avoid any unwanted formation of the  $\alpha$ -anomer a non-participating protecting group, such as a benzyl ether, must be used at the C-2 position of the mannoside donor. Secondly, the axially orientated C-2 functionality sterically hinders the equatorial  $\beta$ -face to attack by the incoming aglycon [19].



**Figure 8.6** Formation of  $\alpha$ -mannosides.



An important analytical consideration when constructing  $\beta$ -mannosides is the determination of the glycosidic bond stereochemistry. The anomeric stereochemistry of both *gluco* and *galacto* pyranosides can be determined by  $^1\text{H}$  NMR spectroscopy by simply measuring the  $^3J_{\text{H-1,H-2}}$  coupling constant. This is not possible in the case of mannopyranosides because the  $^3J_{\text{H-1,H-2}}$  coupling constant for both the  $\alpha$ - and  $\beta$ -anomers is approximately 1–2 Hz, which is due to the *gauche* relationship between the H-1 and H-2 protons [2]. However, it has been shown that the anomeric configuration of  $\alpha$ - and  $\beta$ -mannosides can be accurately differentiated by measuring the  $J_{\text{C-1,H-1}}$  coupling constant; the  $J_{\text{C-1,H-1}}$  values for  $\alpha$ -mannosides are usually  $>170$  Hz whereas the  $J_{\text{C-1,H-1}}$  values for  $\beta$ -mannosides are in the region of  $<160$  Hz [20–22]. Experiments have also provided useful tools for the measurement of the anomeric configuration of mannosides [23].

Despite these difficulties, there have been a number of ingenious solutions towards the synthesis of  $\beta$ -mannosides. This chapter provides the reader with a brief review of the current literature and comprehensively describes a number of experimental procedures, which are specifically directed towards the formation of  $\beta$ -linked mannose containing disaccharides.

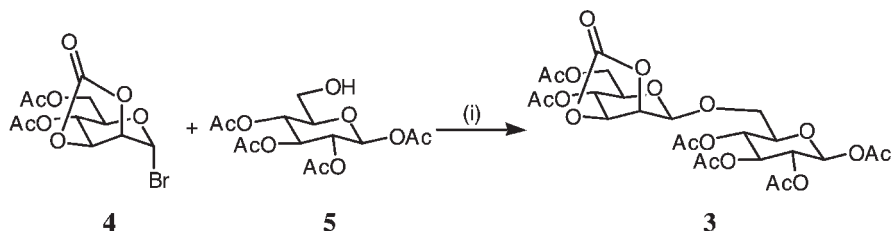
### 8.3 SYNTHETIC METHODS

#### 8.3.1 The use of insoluble promoters

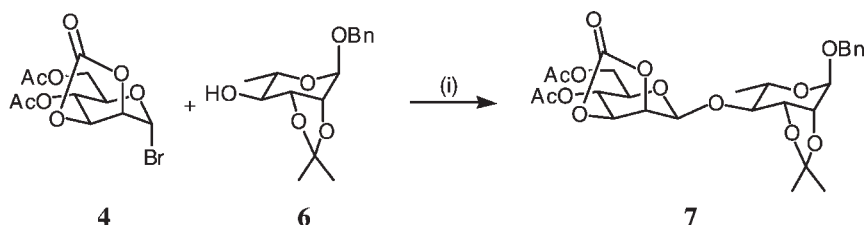
The earliest example of a  $\beta$ -mannoside synthesis was performed by Gorin and Perlin and concentrated on the use of the Koenigs–Knorr insoluble silver salt methodology [24]. The  $\beta$ -mannoside **3** was successfully formed by treatment of the  $\alpha$ -bromo donor **4** with the reactive primary *gluco* acceptor **5** using silver oxide as the promoter (Scheme 8.1). The total yield for the reaction was 45% in favour of the  $\beta$ -mannoside **3**, with only a trace of the  $\alpha$ -anomer being isolated. There are a number of factors that attribute to the successful outcome of this reaction. Firstly, it is believed that the anomeric bromide of the mannoside donor **4** co-ordinates with the bulky insoluble silver salt, thus blocking the  $\alpha$ -face to nucleophilic attack. The absence of a participating protecting group at the C-2 position, combined with the blocked  $\alpha$ -face, results in acceptor **5** displacing the anomeric bromide in an  $\text{S}_{\text{N}}2$  like fashion. Secondly, the carbonate protecting group of donor **4** exerts strain on the pyranose ring effectively disfavours the formation of a planar carbonium ion.

Silver oxide has also been successfully used for the formation of  $\beta$ -mannosyl-rhamnose linkages, which are present in the O-specific polysaccharide of *Salmonella* [17]. Kochetkov and co-workers effectively coupled identical donor **4** with the rhamnose acceptor **6** by activation with silver oxide to exclusively afford the  $\beta$ -Man-(1  $\rightarrow$  4)- $\alpha$ -Rha disaccharide **7** in an impressive 91% yield (Scheme 8.2) [25].

Other first generation insoluble promoters that include silver tosylate [26], silver salicylate [27], silver imidazolate [28] and silver carbonate [29] have proved



**Scheme 8.1** (i) AgO, I<sub>2</sub>, CHCl<sub>3</sub>, rt, 45%.

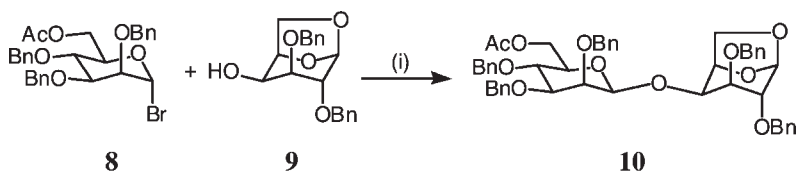


**Scheme 8.2** (i) Ag<sub>2</sub>O, 4 Å mol. sieves, CHCl<sub>3</sub>, rt, 91%.

successful for the synthesis of  $\beta$ -mannosides. However, their use has generally been limited to primary carbohydrate acceptors and reactive non-carbohydrate alcohols. Importantly, coupling at the 4-position of the notoriously unreactive GlcNAc acceptor, to afford the residue present in the core structure of *N*-linked glycoproteins, proved to be unsuccessful with these promoters.

The introduction of the more reactive silver silicate promoter by Paulsen and Lockoff overcame the problem of coupling less reactive secondary carbohydrate acceptors [30]. Activation of the mannosyl bromide donor **8** with silver silicate in the presence of the anhydro acceptor **9** exclusively afforded  $\beta$ -mannoside **10** in an excellent 81% yield (Scheme 8.3).

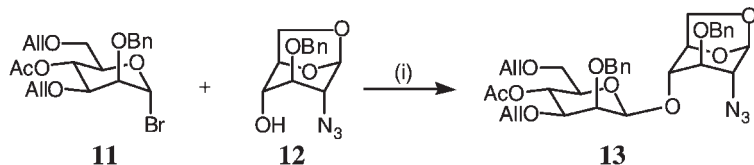
This methodology was further enhanced by the realisation that an acyl protecting group at the C-2 position effectively hinders the formation of a carbonium ion intermediate [31]. This phenomenon was exploited by Paulsen and co-workers in the coupling of mannoside donor **11** with the GlcNAc precursor acceptor **12** by activation with silver silicate, to afford the  $\beta$ -disaccharide **13** in a 65% yield (Method 1) [32].



**Scheme 8.3** (i) Ag silicate, 4 Å mol. sieves DCM, rt, 81%.

*Method 1*

Silver silicate promoted  $\beta$ -mannosylation of bromo mannopyranosyl donors [32].



(i) Ag silicate, 4 Å mol. sieves, DCM, rt, 65%.

*Notes and discussion.* This method allows efficient entry to 4-*O*-(4-*O*-acetyl-3,6-di-*O*-allyl-2-*O*-benzyl- $\beta$ -D-mannopyranosyl)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- $\beta$ -D-glucopyranose by use of the silver silicate catalyst procedure. The target is of use for entry to a branched  $\beta$ -mannose containing pentasaccharide, present within *N*-glycoproteins.

*Materials.*

Donor <b>11</b> [32] (1.4 g, 3.1 mmol)	treat as toxic
Acceptor <b>12</b> [33] (675 mg, 2.4 mmol)	treat as toxic
4 Å molecular sieves (1.3 g)	irritant
Silver silicate [30] (2.2 g, 7.0 mmol)	irritant
Dichloromethane (30 ml)	harmful
Water	non-toxic
MgSO <sub>4</sub>	assume toxic
Celite <sup>®</sup>	harmful
Toluene/acetone for chromatography	flammable, harmful

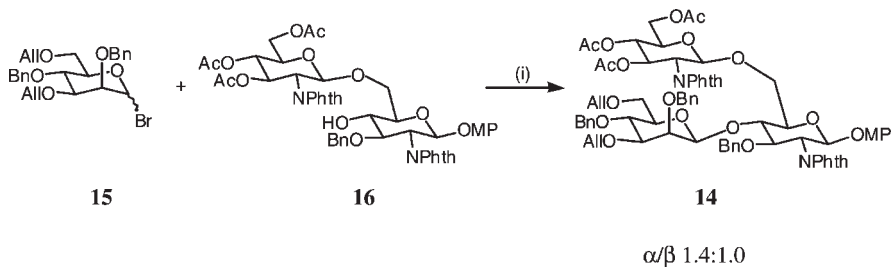
*Equipment.*

- Round bottomed flasks (1 × 100 ml, 1 × 50 ml) with rubber septa and magnetic stirrer bars
- Magnetic stirrer
- Source of dry argon (or nitrogen) gas
- Syringe and needle
- Syringe Pump
- Sinter funnel
- Separating funnel and conical flasks
- Rotary evaporator
- Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

Equip a flame dried round bottomed flask (100 ml) with a magnetic stirrer bar, 4 Å molecular sieves (1.3 g), acceptor **12** (1.4 g, 3.1 mmol) and silver silicate (2.2 g, 7.0 mmol) and purge with argon gas. Add anhydrous dichloromethane (10 ml) and stir the resulting mixture at room temperature under argon for 2 h. Equip a second flame dried round bottom flask (50 ml) with donor **11** (675 mg, 2.4 mmol) and purge with argon gas. Add anhydrous dichloromethane (20 ml) and then add the resulting solution, with the use of a syringe pump, to the initial flask over a 3 h period and continue stirring at room temperature under argon. After a further 2 h, dilute the reaction mixture with dichloromethane (150 ml) and then filter through a Celite<sup>®</sup> pad. Wash the filtrate with water (50 ml), dry the organic phase with MgSO<sub>4</sub>, filter and concentrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a toluene/acetone (40:1) solvent system to give exclusive entry to the β-disaccharide **13** (1.03 g, 65%) as a syrup;  $[\alpha]_{\text{D}}^{20} = -43.3$  (*c* 1.03, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 7.46–7.23 (10H, m, *ArH*), 5.86–5.71 (2H, m, 2 × CH<sub>2</sub>CH=CH<sub>2</sub>), 5.53 (1H, s, H-1), 5.22 (1H, dd, *J* = 9.7 Hz, *J*<sub>4'5'</sub> = 9.4 Hz, H-4'), 5.25–5.09 (4H, m, 2 × CH<sub>2</sub>CH=CH<sub>2</sub>), 4.94 (1H, d, *J* = 12.4 Hz, O-CH<sub>2</sub>Ph), 4.85 (1H, d, *J* = 12.4 Hz, O-CH<sub>2</sub>Ph), 4.73 (1H, s, H-1'), 4.70 (1H, dd, *J* = 1.0, 6.0 Hz, H-5), 4.67 (1H, d, *J* = 11.7 Hz, O-CH<sub>2</sub>Ph), 4.58 (1H, d, *J* = 11.7 Hz, O-CH<sub>2</sub>Ph), 4.16 (1H, dd, *J* = 1.0, 7.3 Hz, H-6a), 3.99 (1H, s, H-4), 3.99 (1H, d, *J* = 2.9 Hz, H-2'), 3.97–3.92 (3H, m, CH<sub>2</sub>CH=CH<sub>2</sub>), 3.79 (1H, dd, *J* = 6.0, 7.3 Hz, H-6b), 3.79 (1H, s, H-3), 3.84–3.78 (1H, m, CHCH=CH<sub>2</sub>), 3.62 (1H, dd, *J* = 7.8, 11.1 Hz, H-6b'), 3.54 (1H, dd, *J'* = 2.9, 11.1 Hz, H-6a'), 3.53 (1H, ddd, *J* = 9.4, 2.9, 7.8 Hz, H-5'), 3.42 (1H, dd, *J* = 2.9, 9.7 Hz, H-3'), 3.24 (1H, s, H-2), 2.08 (3H, s, CH<sub>3</sub>CO); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) 100.70 (d, *J*<sub>C-1,H-1</sub> = 175.3 Hz, C-1), 98.42 (d, *J*<sub>C-1',H-1'</sub> = 154.9 Hz, C-1'); analysis calculated for C<sub>34</sub>H<sub>41</sub>N<sub>3</sub>O<sub>10</sub>: C, 62.66; H, 6.34; N, 6.45. Found: C, 62.53; H, 6.64; N, 6.35%.

Ogawa and co-workers have also utilised this methodology for the synthesis of trisaccharide **14**, which corresponds to an asparagine linked core structure [34]. This was the first report, employing silver silicate, of  $\beta$ -mannosylation to the C-4 hydroxyl group of a GlcNAc acceptor carrying a glycosyl residue. Again, the use of silver silicate successfully coupled mannoside donor **15** with the disaccharide acceptor **16** to afford trisaccharide **14** in a 53% yield (Scheme 8.4). However, it



**Scheme 8.4** (i) Ag silicate, 4 Å mol. sieves DCM,  $-15\text{ }^{\circ}\text{C}$  to rt, 53%.

should be noted that this reaction was generally slow and less efficient affording an anomeric ratio of 1.0:1.4 in favour of the  $\alpha$ -anomer.

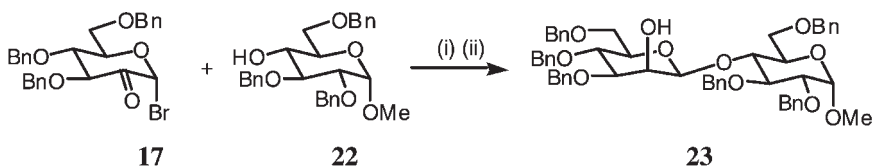
### 8.3.2 The use of 2-oxo glycosyl halides

Access to  $\beta$ -mannosides can be achieved by epimerising the C-2 hydroxyl group of a  $\beta$ -glucoside, however this can be synthetically time consuming [35]. A more efficient alternative to the deprotection, oxidation, reduction methodology was developed by Lichtenthaler and co-workers and focused on the use of 2-oxo bromide **17** as the glycosyl donor [23]. Access to 2-oxo bromide **17** can be achieved from acetobromoglucose **18** in a simple 4-step synthetic sequence with an overall yield of 54% (Scheme 8.5).

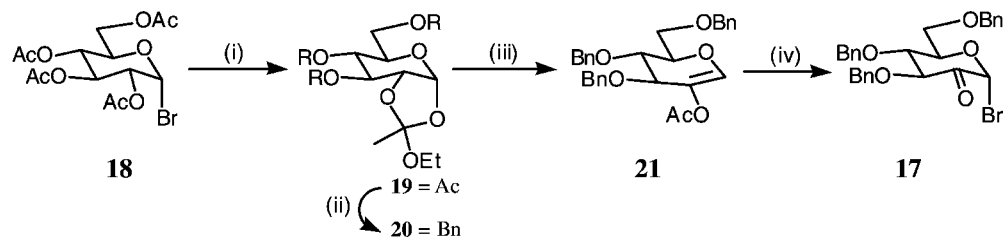
Initial glycosylation studies of the 2-oxo bromide **17** involved the use of silver carbonate as the activator, but good yields and high  $\beta$ -selectivity were only obtained with a variety of primary glycosyl acceptors. The high degree of  $\beta$ -selectivity is attributed to the electron withdrawing effect of the ketone functionality, which is believed to limit the formation of a carbonium ion intermediate. The reduction of the resultant  $\beta$ -linked 2-oxo glycoside with sodium borohydride is highly stereoselective ( $>20:1$ ) in favour of the  $\beta$ -mannoside when the protecting group at the C-3 position is a benzyl ether. Lower yields for the reduction step are observed if the protecting group at the C-3 position is an electron withdrawing acyl group. Lichtenthaler and co-workers utilised this methodology towards the total synthesis of the trisaccharide component of the *Hyriopsis schlegelii* glycosphingolipid [36]. The more reactive silver aluminosilicate promoter developed by van Boeckel [37] was employed for the coupling of the 2-oxo bromide donor **17** with glucosyl acceptor **22**, which proceeded in exclusive  $\beta$ -fashion. Subsequent reduction with sodium borohydride afforded  $\beta$ -mannoside **23** in a very good yield of 81% over the two steps (Method 2).

#### Method 2

Generation of the  $\beta$ -D-mannopyranosidic linkage via  $\text{Ag}^+/\text{SiO}_2\text{--Al}_2\text{O}_3$  activation of 2-ulosyl bromides [36].



(i)  $\text{Ag}^+/\text{SiO}_2\text{--Al}_2\text{O}_3$ , 4 Å mol sieves, DCM, rt, 87%; (ii)  $\text{NaBH}_4$ , DCM/MEOH, 0 °C, 87%.



**Scheme 8.5** (i)  $\text{Bu}_4\text{NBr}$ , collidine, EtOH, 85%; (ii)  $\text{BnBr}$ , KOH, DMF, 81%; (iii) Pyridine,  $\text{C}_6\text{H}_5\text{Br}$ , 156 °C, 85%; (iv) NBS/EtOH, 92%.

*Notes and discussion.* The ulosyl bromide **17** used in this reaction is easily accessed from acetobromoglucose but is of quite low reactivity: under Koenigs–Knorr conditions, or when silver silicate was used as the promoter, the glycosidation reaction was extremely sluggish and considerable amounts of the 1,6-anhydro product derived from bromide **17** formed. Therefore this method necessitates the use of the highly reactive promoter  $\text{Ag}^+/\text{SiO}_2\text{--Al}_2\text{O}_3$ . Ulosyl donors of enhanced reactivity could alternatively be accessed by the treatment of bromide **17** with alkylthio or alkylsulfoxy residues, or with iodine [38]. The method outlined below is representative of the methodology developed by Lichtenhaler and consists of two synthetic steps—a  $\beta$ -glycosidation reaction and a carbonyl reduction transformation. Both steps proceed with excellent stereocontrol.

*Materials.*

Donor <b>17</b> [23] (510 mg, 1.0 mmol)	treat as toxic
Acceptor <b>22</b> [39] (600 mg, 1.3 mmol)	treat as toxic
4 Å molecular sieves (1 g)	irritant
Silver aluminosilicate (1.5 g, 4.8 mmol)	irritant
Dichloromethane	harmful
Methanol	toxic, flammable
Sodium borohydride (350 mg)	avoid contact with water
$\text{Na}_2\text{SO}_4$	assume toxic
1% aq. citric acid solution	assume toxic
Celite <sup>®</sup>	harmful
Water	non-toxic
Chloroform/ethyl acetate and cyclohexane for chromatography	flammable, toxic

*Equipment.*

- Round bottomed flasks (2 × 10 ml, 1 × 250 ml) with rubber septa and magnetic stirrer bars
- Magnetic stirrer
- Source of dry argon (or nitrogen) gas
- Syringe and needle
- Sinter funnel
- Separating funnel and conical flasks
- Rotary evaporator
- Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

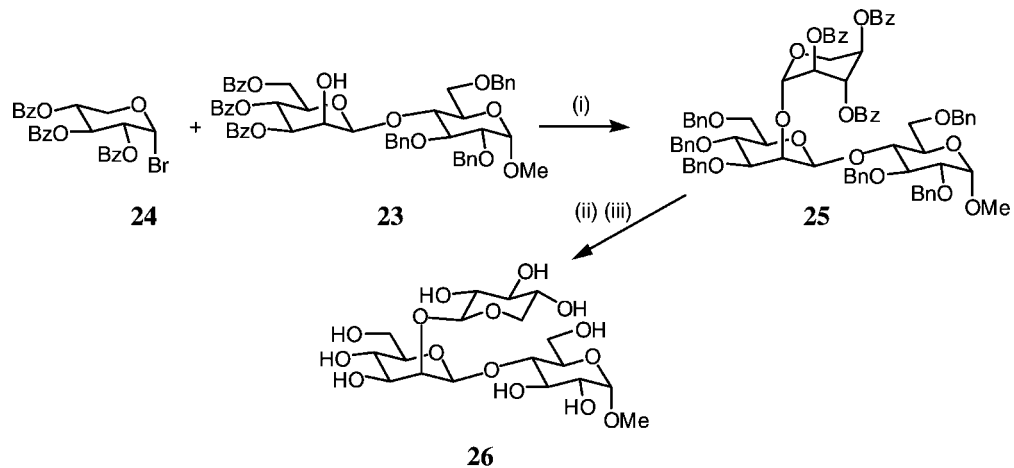
Equip a flame dried round bottomed flask (10 ml) with a magnetic stirrer bar, acceptor **22** (600 mg, 1.3 mmol), 4 Å molecular sieves (1 g), the silver aluminosilicate catalyst (1.5 g, 4.8 mmol) and then purge with argon gas. Add anhydrous dichloromethane (4 ml) and stir at room temperature under argon for 15 min. Equip a second flame dried round bottom flask (10 ml) with donor **17** (510 mg, 1.0 mmol) and purge with argon gas. Add anhydrous dichloromethane (3 ml), and then add the resulting solution in a dropwise manner to the initial reaction flask over a 20 min period. Once the addition is complete, filter the reaction mixture through a Celite® pad, wash the Celite® pad twice with dichloromethane (2 × 50 ml) and concentrate the filtrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a chloroform/ethyl acetate (4:1) solvent system. Dissolve the resulting product in anhydrous dichloromethane (40 ml) under argon and anhydrous methanol (40 ml) in a round bottomed flask (250 ml) and cool to 0 °C. Add sodium borohydride (350 mg) portionwise and continue stirring at 0 °C by external cooling. After 4 h dilute the reaction mixture with dichloromethane (50 ml) and then wash the organic phase with water (2 × 50 ml), followed by 1% aq. citric acid (50 ml) and then with more water (2 × 50 ml). Dry the organic phase (Na<sub>2</sub>SO<sub>4</sub>), filter and concentrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a cyclohexane/ethyl acetate (3:1) solvent system to afford disaccharide **23** as a colourless syrup (730 mg, 81%);  $[\alpha]_D^{20} = +13.6$  (*c* 1.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) *mannosyl-H*: 2.49 (bs, 1H), 3.23 (m, 1H), 3.30 (dd, 1H, *J* = 2.9, 9.2 Hz), 3.64 (m, 1H), 3.76 (m, 1H), 3.84 (dd, 1H, *J* = 9.2, 9.4 Hz), 3.93 (m, 1H), 4.58 (bs, 1H); *glucosyl-H*: 3.35 (s, 1H), 3.50 (dd, 1H, *J* = 3.6, 8.9 Hz), 3.59 (m, 2H), 3.76 (m, 1H), 3.90–4.0 (m, 2H), 4.59 (d, 1H, *J* = 3.6 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) *mannosyl-C*: 67.8, 69.9, 74.3, 75.5, 81.5, 99.8; *glucosyl-C*: 55.2, 69.5, 69.9, 75.6, 79.5, 80.7, 98.1; *m/z* (FAB) 897 (M<sup>+</sup>); analysis calculated for C<sub>55</sub>H<sub>60</sub>O<sub>11</sub>: C, 73.64; H, 6.74. Found: C, 73.54; H, 6.82%.

The advantage of the 2-oxo reduction protocol is that the C-2 hydroxyl group is free for further elaboration, thus avoiding tedious protecting group manipulations. Indeed the synthesis of the target trisaccharide of the *H. schlegelii* glycosphingolipid was simply completed by coupling of the xylose bromide donor **24** onto the C-2 hydroxyl group of disaccharide **23** with silver triflate, which proceeded smoothly in a 71% yield without detectable formation of the α-anomer.† Subsequent two-step protecting group removal afforded the target trisaccharide **26** (Scheme 8.6).

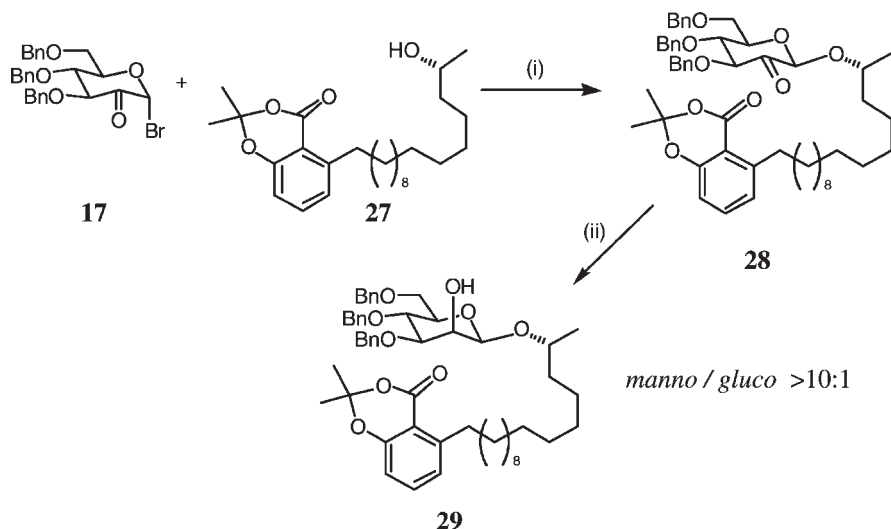
Furstner and Konetzki have successfully adopted this approach for the synthesis of the fungal metabolite **1** [15]. Treatment of 2-oxo bromide donor **17** with alcohol **27** in the presence of aluminosilicate solely afforded β-glycoside **28**, which was reduced without further purification with sodium borohydride. Mannoside **29** was obtained in a 78% yield over two steps with excellent stereoselectivity (Scheme 8.7).

† NMR evidence indicates that the xylose residue of protected trisaccharide **25** exists in a <sup>1</sup>C<sub>4</sub> conformation. In the deprotected trisaccharide **26** the xylose residue adopts the usual <sup>4</sup>C<sub>1</sub> conformation.





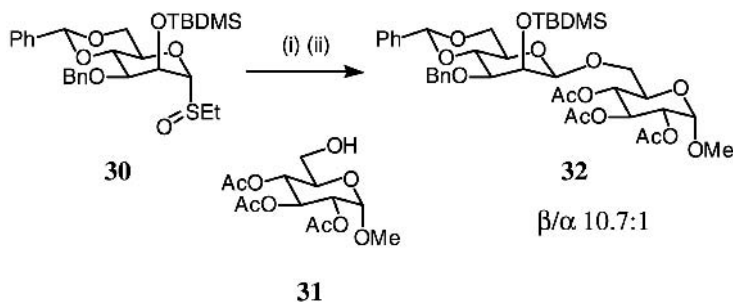
**Scheme 8.6** (i) AgOTf, PhCH<sub>3</sub>/DCM,  $-40^{\circ}\text{C}$ , 71%; (ii) NaOMe/MeOH, rt; (iii) 10% Pd/C, H<sub>2</sub>, MeOH, rt, 82% over two steps.



**Scheme 8.7** (i) Ag silicate, 3 Å mol. sieves, DCM,  $-30^{\circ}\text{C}$ ; (ii)  $\text{NaBH}_4$ , MeOH/DCM,  $-78^{\circ}\text{C}$ , 78% over two steps.

### 8.3.3 $\text{S}_{\text{N}}2$ Displacement of $\alpha$ -mannosyl triflates

Crich and co-workers have developed a highly efficient protocol for the synthesis of  $\beta$ -mannosides based on Kahne and co-workers sulfoxide glycosylation methodology [40]. Initial studies focused on the glycosylation of a sulfoxide donor with primary carbohydrate acceptors using triflic anhydride as the activator [41]. Extensive experimentation indicated that the order of addition of the reactants is crucial for achieving high  $\beta$ -selectivities and synthetically useful yields. An example of this methodology is outlined in Scheme 8.8. Sulfoxide donor **30** and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) were first treated with triflic anhydride in ether/benzene (7:1) at  $-78^{\circ}\text{C}$  followed by the addition of acceptor **31** with slow



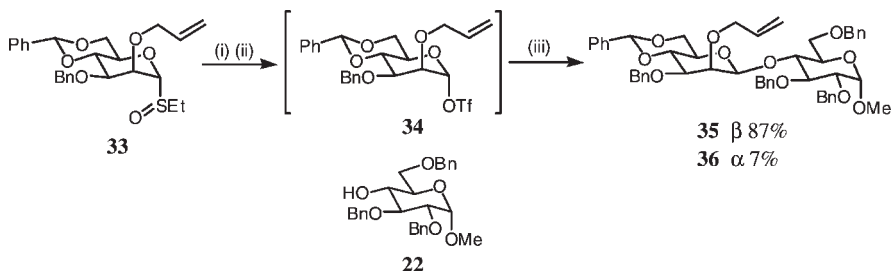
**Scheme 8.8** (i)  $\text{Tf}_2\text{O}$ , DTBMP,  $\text{Et}_2\text{O}/\text{PhH}$ ,  $-78^{\circ}\text{C}$ ; (ii) **31**,  $-78$  to  $0^{\circ}\text{C}$ , 94%.

warming to 0 °C. This procedure afforded disaccharide **32** in a total yield of 94% with a  $\beta/\alpha$  ratio of 10.7:1.

Further investigations determined that the glycosylation of hindered secondary carbohydrate acceptors was also possible using this methodology [42]. It was observed that the presence of the rigid 4,6-*O*-benzylidene protecting group is crucial for high  $\beta$ -selectivities as it effectively hinders the formation of an anomeric carbonium ion. Yields and  $\beta$ -selectivities were further improved by reducing the steric bulk at the C-2 position of the sulfoxide donor and by changing the solvent to dichloromethane. Crich and co-workers successfully applied their protocol to the synthesis of the trisaccharide component of the *H. schlegelii* glycosphingolipid [43]. Treatment of sulfoxide donor **33** with DTBMP, followed by triflic anhydride, results in the formation of the anomeric triflate **34**, whose formation was observed by low temperature NMR experiments [44]. Subsequent addition of the secondary acceptor **22** results in  $S_N2$  displacement of the anomeric triflate to afford  $\beta$ -disaccharide **35** in an excellent yield of 87%. A minor quantity of the corresponding  $\alpha$ -disaccharide **36** was also observed (Method 3) [45].

### Method 3

Direct synthesis of  $\beta$ -mannoside **35** from glycosyl sulfoxide **33** [45].



(i) DTBMP, DCM,  $-78\text{ }^{\circ}\text{C}$ ; (ii)  $\text{Ti}_2\text{O}$ ,  $-78\text{ }^{\circ}\text{C}$ ; (iii) **22**, DCM,  $-78\text{ }^{\circ}\text{C}$  to rt.

**Notes and discussion.** The sulfoxide donor **33** is easily prepared, as a single diastereomer, by treatment of the analogous thioethyl donor [46] with oxone in aqueous methanol. The key mannosylation reaction is achieved by activation of the sulfoxide with triflic anhydride in dichloromethane at  $-78\text{ }^{\circ}\text{C}$  in the presence of DTBMP.

### Materials.

Donor <b>33</b> [45,46] (428 mg, 0.93 mmol)	treat as toxic
Acceptor <b>22</b> [47] (818 mg, 1.83 mmol)	treat as toxic
Triflic anhydride (173 $\mu\text{l}$ , 1.02 mmol)	corrosive
2,6-Di- <i>tert</i> -butyl-4-methyl pyridine (DTBMP) (377 mg, 1.83 mmol)	harmful
Dichloromethane (35 ml)	harmful

Saturated aq. NaHSO <sub>3</sub>	assume toxic
MgSO <sub>4</sub>	assume toxic
Brine solution	assume toxic
Hexane/ethyl acetate for chromatography	flammable, toxic

#### *Equipment.*

Round bottomed flasks (1 × 100 ml, 1 × 25 ml) with rubber septa and magnetic stirrer bars  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) gas  
 Reaction dewar and dry ice  
 Syringe and needle  
 Sinter funnel  
 Separating funnel  
 Rotary evaporator  
 Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

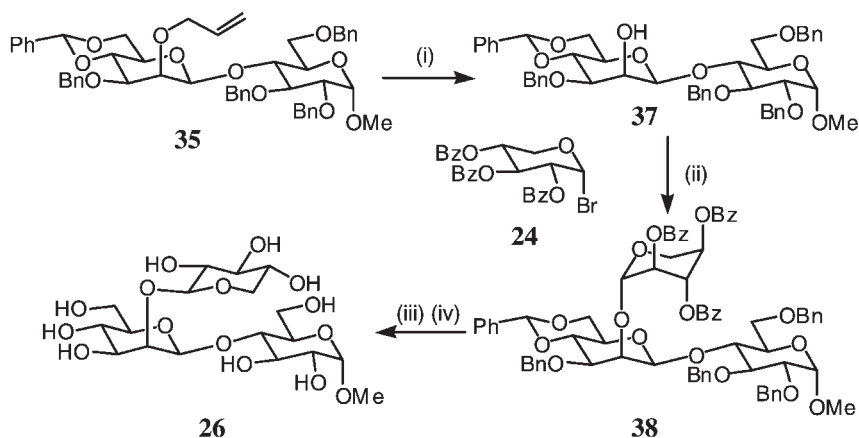
Equip a flame dried round bottomed flask (100 ml) with a magnetic stirrer bar, donor **33** (428 mg, 0.93), DTBMP (377 mg, 1.83 mmol) and purge with argon gas. Add anhydrous dichloromethane (25 ml) and reduce the temperature to  $-78^{\circ}\text{C}$  by external cooling. Add triflic anhydride (1.73  $\mu\text{l}$ , 1.02 mmol) and stir the resulting reaction mixture at  $-78^{\circ}\text{C}$  under argon for 5 min. Equip a second flame dried round bottom flask (25 ml) with acceptor **22** (818 mg, 1.83 mmol) and purge with argon gas. Add anhydrous dichloromethane (10 ml), add the resulting solution in a dropwise manner to the initial flask and continue stirring at  $-78^{\circ}\text{C}$  under argon for a further 1 h. Allow the reaction mixture to warm to  $0^{\circ}\text{C}$  and then dilute the reaction mixture with dichloromethane (150 ml). Wash the organic phase with saturated aq. NaHSO<sub>3</sub> (30 ml), brine solution (30 ml) and then dry with MgSO<sub>4</sub>, filter and concentrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a hexane/ethyl acetate (20:1) solvent system to give the  $\beta$ -disaccharide **35** (731 mg, 87%) as the major product;  $[\alpha]_{\text{D}}^{20} = -12.1$  (*c* 1.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 3.05 (m, 1H), 3.32 (dd, *J* = 3.5, 9.7 Hz, 1H), 3.40 (s, 3H), 3.51–3.80 (m, 5H), 3.88–3.96 (m, 2H), 4.00–4.10 (m, 2H), 4.29 (m, 2H), 4.35 (d, *J* = 11.8 Hz, 2H), 4.60–4.70 (m, 4H), 4.76–4.86 (m, 3H), 5.11 (d, *J* = 10.5 Hz, 2H), 5.13 (d, *J* = 11.4 Hz, 1H), 5.28 (d, *J* = 18.4 Hz, 1H), 5.52 (s, 1H), 5.90–6.04 (m, 1H), 7.20–7.50 (m, 25H); <sup>13</sup>C (75 MHz, CDCl<sub>3</sub>) 55.3, 67.1, 68.2, 68.5, 69.5, 72.4, 73.5, 74.4, 75.2, 77.0, 77.4, 77.8, 78.6, 78.9, 80.1, 98.3, 101.2, 116.8, 125.9, 127.3, 127.5, 127.7, 128.0, 128.3, 128.5, 128.8, 135.4, 136.5, 137.5, 138.0, 139.3; analysis calculated for C<sub>51</sub>H<sub>56</sub>O<sub>11</sub>·1H<sub>2</sub>O: C, 70.97; H, 6.77. Found: C, 70.93; H, 6.64%; and the corresponding  $\alpha$ -disaccharide **36** as the minor product (60 mg, 7%);  $[\alpha]_{\text{D}}^{20} = +21$  (*c* 3.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 3.39 (s, 3H), 3.55 (dd, *J* = 3.5, 9.7 Hz,

1H), 3.65–3.75 (m, 5H), 3.75–4.00 (m, 7H), 4.10 (m, 1H), 4.18 (m, 1H), 4.50–4.75 (m, 7H), 4.80 (d,  $J = 12.0$  Hz, 1H), 5.00 (d,  $J = 10.3$  Hz, 1H), 5.06 (d,  $J = 17.3$  Hz, 1H), 5.11 (d,  $J = 11.7$  Hz, 1H), 5.59 (s, 1H), 5.66 (m, 1H), 7.20–7.55 (m, 25H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ) 55.3, 65.0, 68.6, 68.8, 69.5, 72.7, 72.9, 73.1, 73.5, 75.8, 76.0, 77.0, 77.4, 78.9, 79.9, 81.4, 97.7, 101.3, 101.6, 116.8, 126.0, 126.7, 127.4, 127.6, 127.9, 128.0, 128.2, 128.3, 128.4, 128.7, 134.7, 137.7, 137.9, 138.5.

The *O*-allyl protecting group at the C-2 position of disaccharide **35** was removed by treatment with potassium *tert*-butoxide followed by catalytic oxidation with  $\text{OsO}_4$  and an excess of *N*-methylmorpholine *N*-oxide (NMNO), as overall oxidant, leading to the formation of the acceptor disaccharide **37** in 60% yield. Xylosylation was successfully achieved by silver triflate activation of the xylose bromide donor **24** and coupling with acceptor **37**. This afforded exclusive entry to the  $\beta$ -anomer **38** in a 68% yield (90% when based on recovered starting material). A simple two-step protecting group removal procedure furnished the target trisaccharide **26** in an overall yield of 95% (Scheme 8.9). The  $^{13}\text{C}$  NMR and optical rotation of trisaccharide **26** were identical to those reported by Lichtenthaler (*vide supra*) [36]. Crich and co-workers have also successfully applied their methodology towards the synthesis of complex  $\beta$ -mannan structures [48] and the mannosyl erythritol lipid MEL A [49].

### 8.3.4 Dibutylstannylene complexes

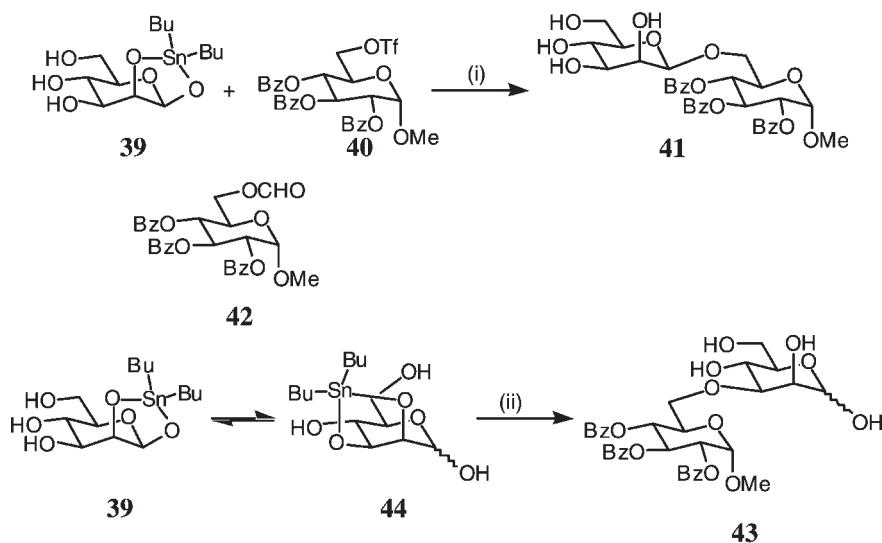
Kováč and Hodosi have applied stannylene chemistry for the synthesis of  $\beta$ -mannosides [50]. The use of 1,2-*O*-*cis*-stannylene acetals, first developed by



**Scheme 8.9** (i)  $\text{KO}^t\text{Bu}$ , DMSO, 90 °C then  $\text{OsO}_4$ , NMNO, 80% aq. acetone, rt, 60%; (ii) DTBMP,  $\text{AgOTf}$ , DCM,  $-40$  to  $0$  °C, 68%; (iii) NaOMe, MeOH, 90%; (iv)  $\text{H}_2$ , Pd/C, MeOH, 95%.

Schuerch and co-workers [51], effectively reverses the roles of the glycosyl donor and acceptor. Kovác and Hodosi have exploited this phenomenon by employing a 1,2-*O*-stannylene mannose derivative as a powerful nucleophile to displace, via the  $S_N2$  process, suitable leaving groups in carbohydrates. Advantages of this methodology include the absence of carbonium ion formation and minimal hydroxyl group protection of the glycosyl donor. The stereospecific formation of a  $\beta$ -mannoside linkage is attributed to the favoured *cis*-arrangement of carbohydrate derived 1,2-*O*-stannylene acetals. Initial studies were performed using DMF as a solvent. For example, the reaction of stannylene acetal **39** with the C-6 triflate *gluco* derivative **40** in DMF afforded the desired  $\beta$ -mannoside **41** in a 40% yield. However, side reactions were sometimes observed, which included the formation of formates, such as **42**, and ether linked disaccharides, such as **43**. The latter is attributed to the isomerisation of stannylene acetal **39** to **44** during the reaction. These side reactions could be minimised by changing the solvent to acetonitrile, which results in the formation of  $\beta$ -disaccharide **41** in 57% yield (Scheme 8.10).

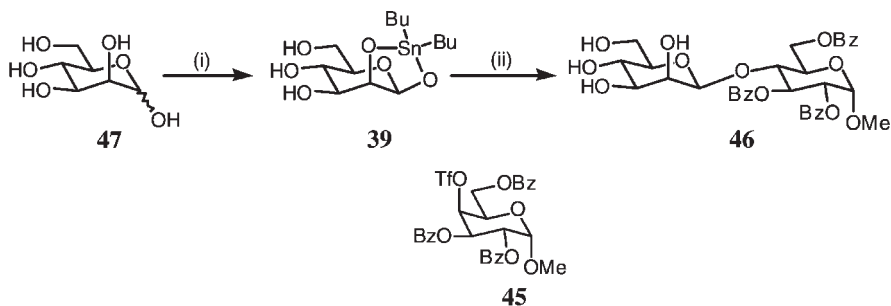
Treatment of the less reactive secondary *galacto* triflate **45** with stannylene **39** also resulted in  $\beta$ -mannoside formation. Once again yields with DMF as a solvent were low and the long reaction time led to unwanted decomposition of starting materials. Optimum yields were obtained by changing the solvent to DMSO, which resulted in the stereoselective formation of  $\beta$ -mannoside **46** in a very good yield of 59% (Method 4) [52].



**Scheme 8.10** (i) DMF, 0–20 °C, 40%; (ii) CH<sub>3</sub>CN, Bu<sub>4</sub>NF, 25 °C, 57%.

## Method 4

Synthesis of  $\beta$ -mannopyranosides using *cis*-1,2-stannylene acetals [50].



(i) Dibutyl tin oxide, MeOH, 60 °C; (ii) **45**, 4 Å mol. sieves, DMSO, rt, 59%.

**Notes and discussion.** In this method a *cis*-1,2-stannylene acetal, derived from mannose, nucleophilically displaces a triflate from the C-4 position of a galactopyranoside, with inversion of stereochemistry. The example reported below allows entry to a Man- $\beta$ -(1,4)-Glu derivative. It has been found that the reactivity of the electrophile and the nucleophilicity of the oxygens within the *cis*-1,2-stannylene acetal greatly affect the overall outcome of the reaction. The nucleophilicity of the oxygens within the stannylene complex decreases in the order: equatorial anomeric > equatorial non-anomeric > axial anomeric. Moreover, the yield and ratio of products are found to be highly solvent dependent: as the solvent polarity increases, the reactivity of the reactants and the yield of the desired  $S_N2$  reactions increase. Thus when the reaction described herein was performed at 25 °C, in DMF, for 5 days, the disaccharide **46** formed in 40%, when it was performed at 25 °C in DMA for 3 days, 53% of the required disaccharide formed, but best yields and selectivities (59%) were obtained by performing the reaction at 25 °C, in DMSO, for 2 days.

## Materials.

Mannose <b>47</b> (360 mg, 2.0 mmol)	assume toxic
Triflate <b>45</b> [53] (200 mg, 0.35 mmol)	treat as toxic
4 Å molecular sieves (200 mg)	irritant
Dibutyl tin oxide (470 mg, 1.9 mmol)	harmful
Methanol	toxic, flammable
Cesium fluoride (290 mg, 1.9 mmol)	harmful
Celite <sup>®</sup>	harmful
Dimethyl sulfoxide	toxic
Dichloromethane/methanol for chromatography	flammable, toxic

*Equipment.*

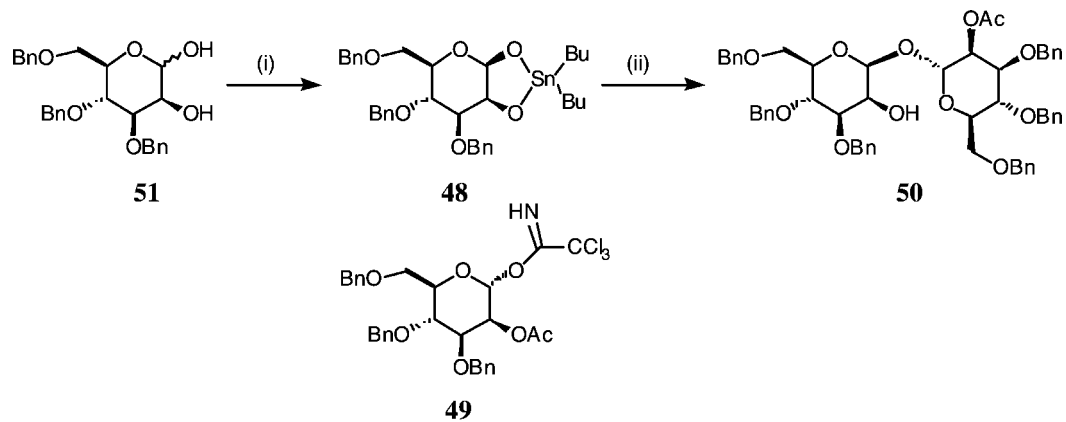
Round bottomed flask (1 × 50 ml) with rubber septum and magnetic stirrer bar  
Magnetic stirrer/hot plate, oil bath  
Source of dry argon (or nitrogen) gas  
Syringe and needle  
Drying pistol and vacuum pump  
Sinter funnel  
Rotary evaporator  
Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

Equip a flame dried round bottomed flask with a magnetic stirrer bar, mannose **47** (360 mg, 2.0 mmol), tributyl tin oxide (470 mg, 1.9 mmol) and then purge with argon gas. Add anhydrous methanol (13 ml) and stir the resulting reaction mixture at 60 °C under nitrogen. After 1.6 h (or until a clear solution is obtained) add cesium fluoride (290 mg, 1.9 mmol) and toluene (2 ml) and concentrate *in vacuo*. Dry the resulting residue in a drying pistol at 50 °C under reduced pressure for approximately 2.5 h. When the residue is dry add anhydrous dimethyl sulfoxide (1.5 ml) and 4 Å molecular sieves (200 mg) and stir at room temperature. After 30 min add the *galacto* triflate **45** (200 mg, 0.35 mmol) and continue stirring vigorously at room temperature. After 2 days concentrate the reaction mixture *in vacuo*, add acetonitrile (150 ml) and filter the resulting suspension through a Celite® pad. Wash the Celite® pad twice with further acetonitrile (2 × 20 ml) and concentrate the filtrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a dichloromethane/methanol solvent system to give disaccharide **46** as a white crystalline solid (120 mg, 59%); mp 106–107 °C (ethanol); [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +104 (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 6.00 (1H, t, *J* = 9.6 Hz, H-3), 5.23 (1H, dd, *J* = 3.6, 10.2 Hz, H-2), 5.13 (1H, d, H-1), 4.70 (1H, bd, H-6a), 4.61 (1H, dd, *J* = 4.2, 12.3 Hz, H-6b), 4.58 (1H, s, H-1'), 4.28 (1H, m, H-5), 4.12 (1H, t, *J* = 9.4 Hz, H-4), 3.95 (1H, d, *J* = 2.5 Hz, H-2'), 3.56 (1H, t, *J* = 9.4 Hz, H-4'), 3.41 (3H, s, OCH<sub>3</sub>), 3.34 (1H, dd, *J* = 9.4 Hz, H-3'), 3.17 (1H, dd, *J* = 3.5, 12.3 Hz, H-6a'), 3.04 (1H, dd, *J* = 2.4 Hz, H-6b'), 2.83 (1H, dbt, H-5'); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 100.55 (C-1', *J*<sub>C,H</sub> = 156.3 Hz), 96.79 (C-1, *J*<sub>C,H</sub> = 176.1 Hz), 76.87 (C-4), 76.04 (C-5'), 73.55 (C-3'), 71.80 (C-2), 71.17 (C-3), 70.89 (C-2'), 68.38 (C-5), 65.79 (C-4'), 63.08 (C-6), 60.51 (C-6'), 55.40 (OCH<sub>3</sub>); *m/z* (CI) 686 (*M* + NH<sub>4</sub><sup>+</sup>); analysis calculated for C<sub>34</sub>H<sub>36</sub>O<sub>14</sub>·0.5H<sub>2</sub>O: C, 60.26; H, 5.50. Found: C, 60.41; H, 5.52%.

The coupling of two mannosyl glycosides through both anomeric centres is also possible with stannylyne chemistry, as demonstrated by Nicolaou and co-workers [14]. One of the most challenging features of the total synthesis of everninomicin 13,384-1 was the formation of a 1β-1α mannoside unit, which required the simultaneous control of the stereochemistry at two anomeric centres. This was accomplished by Nicolaou and co-workers by performing a stereocontrolled reaction between the benzylated stannylyne glycoside **48** and a trichloroacetimidate donor **49**. Disaccharide **50** was successfully formed stereoselectively in a good yield of 66% from the trichloroace-





**Scheme 8.11** (i)  $\text{Bu}_2\text{SnO}$ ,  $\text{MeOH}$ , reflux; (ii) **49**,  $\text{TMSOTf}$ ,  $\text{Et}_2\text{O}$ ,  $0-25^\circ\text{C}$ , 66%.

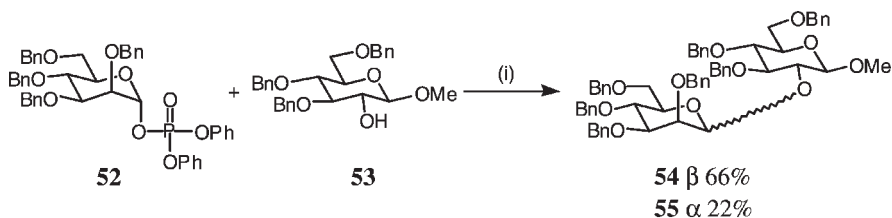
timidate donor **49** and stannylene **48**, using trimethylsulfonyl triflate (TMSOTf) as a Lewis acid activator (Scheme 8.11).

### 8.3.5 The use of glycosyl phosphate donors

Glycosyl phosphates are key intermediates in the biosynthesis of oligosaccharides and have also been utilised as glycosyl donors in the chemical synthesis of glycosidic bonds [54]. Under controlled reaction conditions and with careful choice of solvent, Seeberger and co-workers have applied glycosyl phosphate chemistry towards the formation of  $\beta$ -mannosides [55]. Glycosyl phosphate donor **52**, with a non-participating protecting group at the C-2 position, was successfully coupled to acceptor **53**, with 1.3 equiv. of TMSOTf as an activator. The  $\beta$ -disaccharide **54** was preferentially formed over the  $\alpha$ -disaccharide **55** ( $\alpha/\beta$  1:3) when dichloromethane was employed as a solvent, in a yield of 66% (Method 5).

#### Method 5

Synthesis of a Man- $\beta$ -(1,2)-Glu disaccharide via the acid catalysed coupling of a glycosyl phosphate with a C-2 glucosyl acceptor [55].



(i) TMSOTf, DCM,  $-78^{\circ}\text{C}$ , 88%.

*Notes and discussion.* This method allows direct entry to a Man- $\beta$ -(1,2)-Glu disaccharide under mild reaction conditions. The solvent used for the reaction has a great influence on the stereoselectivity of the reaction. As noted above, dichloromethane is a good choice of solvent for accessing the  $\beta$ -linked product. Interestingly, when the reaction is performed in acetonitrile the major product observed was the corresponding  $\alpha$ -anomer **55** ( $\alpha/\beta$  5.5:1). Attempts to increase the  $\beta$ -selectivity, by employing a 4,6-*O*-benzylidene protected derivative, proved to be unsuccessful. Partial hydrolysis of the benzylidene ring was observed due to the acidic conditions required for glycosyl phosphate activation.

#### Materials.

Donor **52** [55] (36.4 mg, 47  $\mu\text{mol}$ )

treat as toxic

Acceptor **53** [55] (17.4 mg, 39.1  $\mu\text{mol}$ )

treat as toxic

Trimethylsilyl triflate (9.4  $\mu\text{l}$ , 51  $\mu\text{mol}$ )

harmful, corrosive

Toluene	harmful, flammable
Dichloromethane	harmful
Triethylamine	flammable, harmful
Hexane/ethyl acetate for chromatography	flammable, harmful

### Equipment.

Round bottomed flask (1  $\times$  25 ml) with rubber septum and magnetic stirrer bar  
 Magnetic stirrer  
 Source of dry argon (or nitrogen) gas  
 Reaction dewar and dry ice  
 Syringe and needle  
 High vacuum line  
 Rotary evaporator  
 Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

Equip a flame dried round bottomed flask with a magnetic stirrer bar, donor **52** (36.4 mg, 47  $\mu$ mol), acceptor **53** (17.4 mg, 39.1  $\mu$ mol) and then dry the reactants azeotropically with toluene (3  $\times$  5 ml), and leave to dry for 1 h on a high vacuum line. Add anhydrous dichloromethane (2 ml) and stir the resulting reaction mixture at  $-75^\circ\text{C}$  under argon. After 15 min add trimethylsilyl triflate (9.4  $\mu$ l, 51  $\mu$ mol) dropwise and continue stirring at  $-78^\circ\text{C}$  under argon. After a further 30 min add triethylamine (2 equiv.) and allow the reaction mixture to reach room temperature. When room temperature has been obtained, concentrate the reaction mixture *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a hexane/ethyl acetate (3:1) solvent system to give  $\beta$ -disaccharide **54** as a colourless syrup (66%);  $[\alpha]_{\text{D}}^{24} = -24.2$  (*c* 0.67,  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}$  (thin film) 3029, 2864, 1490, 1453, 1362  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) 3.33 (dd,  $J = 2.8, 9.5$  Hz, 1H), 3.42–3.44 (m, 1H), 3.52–3.56 (m, 1H), 3.64–3.84 (m, 8H), 3.93 (a-t,  $J = 9.5$  Hz, 1H), 4.34–4.41 (m, 2H), 4.44 (d,  $J = 7.3$  Hz, 1H), 4.74–4.79 (m, 2H), 4.88–4.90 (m, 3H), 7.17–7.43 (m, 35H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) 56.9, 69.0, 69.7, 71.8, 74.0, 74.5, 75.0, 75.1, 75.3, 75.4, 76.3, 78.3, 80.6, 83.2, 85.5, 101.8, 102.9, 127.0, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 138.1, 138.4, 138.5, 138.9, 139.1;  $m/z$  (FAB) HRMS ( $\text{M}^+$ ) calculated for  $\text{C}_{62}\text{H}_{66}\text{O}_{11}$  986.4065. Found 986.4067; and the corresponding  $\alpha$ -disaccharide **55** as a colourless syrup (22%);  $[\alpha]_{\text{D}}^{24} = +30.6$  (*c* 0.77,  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}$  (thin film) 3029, 2863, 1495, 1453  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) 3.44–3.78 (m, 9H), 3.84–3.86 (m, 1H), 3.90–3.97 (m, 2H), 4.13–4.18 (m, 2H), 4.36 (d,  $J = 11.9$  Hz, 1H), 4.51–4.84 (m, 13H), 4.92 (d,  $J = 11.0$  Hz, 1H), 5.52 (d,  $J = 1.5$  Hz, 1H), 7.10–7.45 (m, 35H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) 57.0, 68.8, 68.9, 72.1, 72.2, 73.4, 73.7, 74.3, 74.9, 75.2, 76.1, 78.5, 79.9, 83.7, 97.6, 104.7, 127.5, 127.6, 127.7, 127.9, 128.0,

128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 138.1, 138.3, 138.7, 138.8, 139.2;  $m/z$  (FAB) HRMS ( $M^+$ ) calculated for  $C_{62}H_{66}O_{11}$  986.4065. Found 986.4069.

### 8.3.6 Intramolecular aglycon delivery

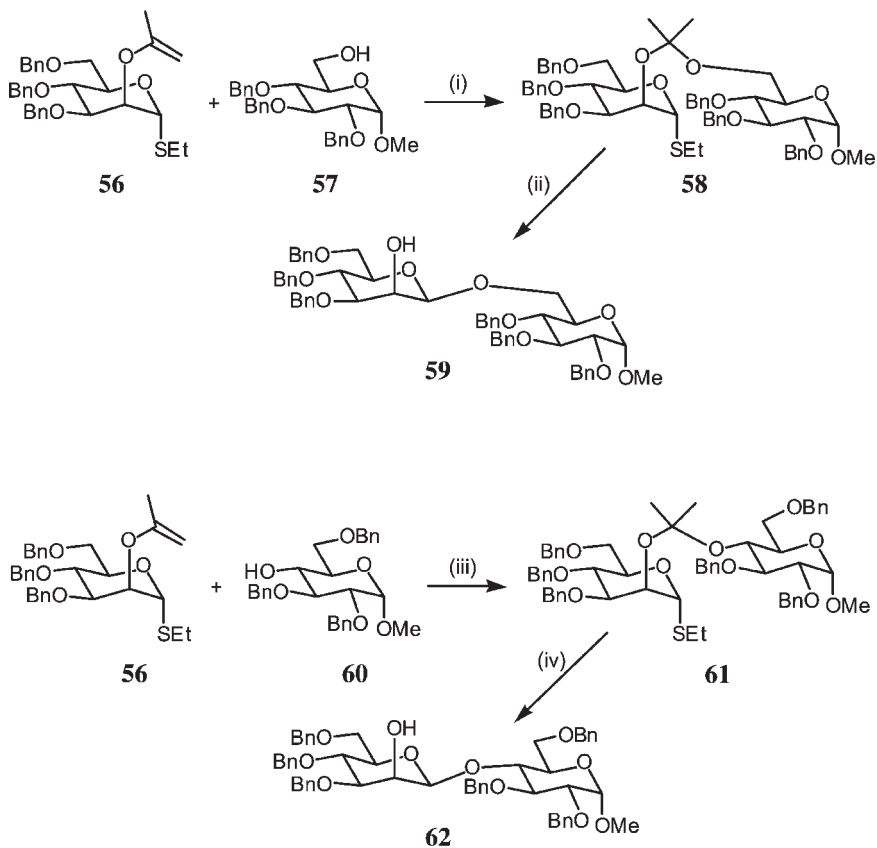
The methods discussed so far have been of an intermolecular nature and have relied on controlled reaction conditions and reagents to achieve high levels of  $\beta$ -selectivity. A novel approach, first introduced by the laboratories of Hindsgaul [22] and Stork [56, 57] involves an intramolecular glycosylation reaction. This methodology, known as intramolecular aglycon delivery (IAD), tethers the glycosyl acceptor to the glycosyl donor in such an orientation that allows the glycosylation to proceed in a stereoselective manner.

The IAD method of Hindsgaul and Barresi employed a mixed acetal tethering system [22]. Tethering of the glycosyl acceptor to the glycosyl donor was achieved by treating enol ether **56** with catalytic  $p$ TSA in the presence of primary acceptor **57** and afforded the mixed acetal adduct **58** in a 51% yield. Subsequent treatment of the mixed acetal **58** with 5 equiv. of NIS over a 16 h period formed the  $\beta$ -disaccharide **59** in a 61% yield. It was later found that addition of the hindered base DTBMP enhanced the yield of the glycosylation step. Indeed, in the case of less reactive acceptors such as the OH-4 acceptor **60**, the yield for the glycosylation step increased from 42 to 77%. It has been postulated that DTBMP may prevent premature decomposition of the acid sensitive mixed acetal tether prior to glycosylation. In both cases the reactions proceeded stereoselectively with no formation of the corresponding  $\alpha$ -mannosides being observed (Scheme 8.12).

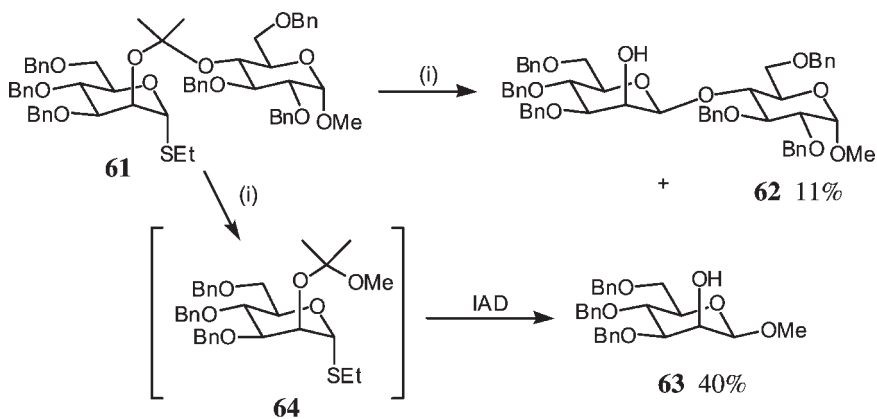
In order to determine the intramolecular nature of the glycosylation step, activation of mixed acetals **58** and **61** were performed in the presence of 1 equiv. of methanol as a competing aglycon. When acetal **58** was treated with NIS and methanol the yield of  $\beta$ -mannoside **59** was unaffected and notably no formation of the methyl mannoside was observed. This observation indicates that the intramolecular glycosylation step is in fact a concerted process, with no formation of a carbonium ion intermediate. Acetal **61** was subjected to the same competing reaction conditions, however the yield of  $\beta$ -mannoside **62** was significantly reduced to 11%. The main product isolated was in fact methyl  $\beta$ -mannoside **63** in a yield of 40%. However, the  $\beta$ -configuration of **63** suggests that this too was formed by intramolecular delivery and its formation is attributed to *trans*-acetalisation of acetal **61** with methanol to form acetal **64** (Scheme 8.13).

Although this methodology provides access to anomERICALLY pure  $\beta$ -mannosides, the experimental procedure is complex and when applied to more unreactive acceptors undesirable side reactions can occur.

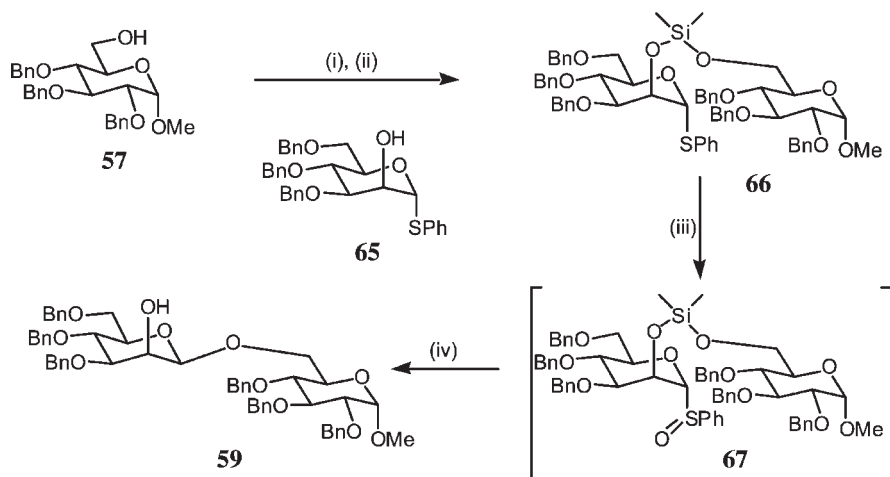
Stork and co-workers investigated the use of silyl acetals as tethers for the stereoselective formation of  $\beta$ -mannosides [56]. Initial studies were performed on primary acceptors of glucose **57** and mannose. Glucosyl acceptor **57** is first converted to its chlorodimethyl ether derivative by treatment with *n*-butyllithium



**Scheme 8.12** (i) cat. *p*TSA, DCM,  $-40^\circ\text{C}$ , 51%; (ii) NIS (5 equiv.), DCM,  $-5^\circ\text{C}$  to rt, 61%; (iii) cat. *p*TSA, DCM,  $-40^\circ\text{C}$ , 57%; (iv) NIS (5 equiv.), DTBMP, DCM  $-5^\circ\text{C}$  to rt, 77%.



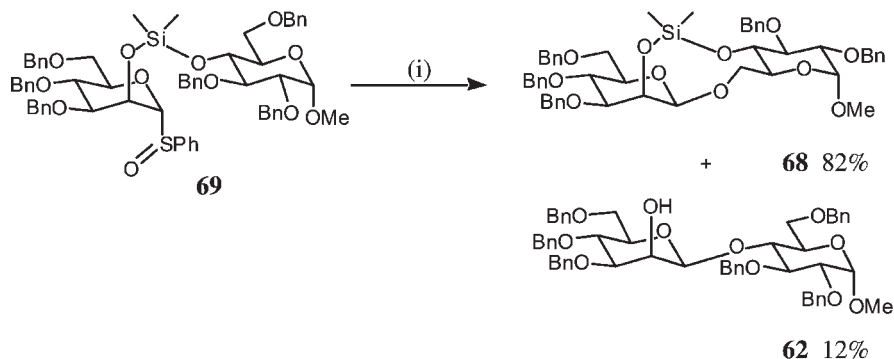
**Scheme 8.13** (i) NIS (5 equiv.), MeOH (1 equiv.), DCM,  $0^\circ\text{C}$  to rt.



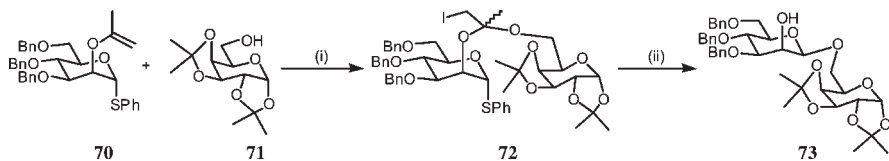
**Scheme 8.14** (i)  $\text{Me}_2\text{SiCl}_2$ , BuLi, THF,  $-78^\circ\text{C}$ ; (ii) **65**, imidazole, THF, rt; (iii) *m*CPBA, DCM,  $-25$  to  $0^\circ\text{C}$ ; (iv)  $\text{TiF}_2\text{O}$ , DTBMP,  $-78^\circ\text{C}$  to rt, 73%.

and dichlorodimethylsilane. Subsequent addition of thioglycoside **65** gave the desired silyl acetal **66** in almost quantitative yield. Oxidation to the sulfoxide **67** by *m*CPBA and subsequent activation with triflic anhydride and DTBMP resulted in intramolecular delivery of the aglycon to afford  $\beta$ -mannoside **59** in a 73% yield (Scheme 8.14).

In later work, this methodology was extended to incorporate secondary acceptors [57].  $\beta$ -Mannosides from C-2 and C-3 glucosyl acceptors were obtained in excellent yields. It was found that higher yields were obtained by coupling the acceptor directly to the sulfoxide donor, thus avoiding manipulations of the sensitive silyl acetal tether. However, a very low yield of 12% was obtained for the formation of 4-*O*-glucosyl  $\beta$ -mannoside **62**. The major product still had the silyl tether intact and was found to be disaccharide **68**. It was assumed that debenzoylation of the C-6 hydroxyl had occurred allowing formation of the observed 1,6-linkage (Scheme 8.15).



**Scheme 8.15** (i)  $\text{TiF}_2\text{O}$ , DTBMP, DCM,  $-100^\circ\text{C}$  to rt.



**Scheme 8.16** (i) NIS (3 equiv.), THF,  $-78\text{ }^{\circ}\text{C}$  to rt, 95%; (ii) NIS (5 equiv.), DTNMP, DCM,  $0\text{ }^{\circ}\text{C}$  to rt, 63%.

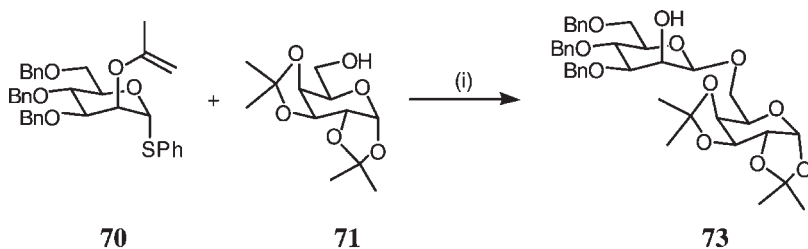
A modification to Hindsgaul's mixed acetal methodology, reported by Fairbanks and co-workers, resulted in the development of a 'one pot' procedure whereby tethering and IAD were achieved in a single manipulation [58]. This methodology utilised *N*-iodosuccinimide (NIS) as an alternative electrophile for the tethering of the aglycon to the glycosyl donor. Initial studies focused on a two-step procedure. Using THF as a solvent, the tethering of diacetone galactose **71** to enol ether **70** with NIS proceeded in an excellent yield of 95%. The mixed acetal intermediate **72**, which was isolated, was then treated with further NIS in the presence of the base DTBMP in dichloromethane to afford  $\beta$ -mannoside **73** in a yield of 63%. No formation of the corresponding  $\alpha$ -mannoside was observed (Scheme 8.16).

Attention was then directed towards the development of a 'one pot' procedure. The success of the tethering reaction with NIS indicated a reactivity differential between the enol ether and the anomeric thiophenyl group. Choice of solvent proved to be crucial, with 1,2-dichloroethane giving the best results.

Enol ether **70** and 3 equiv. of diacetone galactose **71** were treated with NIS in dichloroethane at  $-40\text{ }^{\circ}\text{C}$ . When tethering was complete, as indicated by thin layer chromatography, a further 2 equiv. of NIS and DTBMP were added to the reaction flask. After 2 days the reaction mixture was treated with acidic ion exchange resin to solely afford the  $\beta$ -mannoside **73** in an excellent yield of 84% (Method 6) [59]. As with Hindsgaul's methodology, competition experiments confirmed the intramolecular nature of the reaction.

#### Method 6

NIS-mediated intramolecular aglycon delivery for entry to a Man- $\beta$ -(1,6)-Gal residue [59].



(i) NIS, DTBMP,  $-40\text{ }^{\circ}\text{C}$  to rt, then Dowex  $\text{H}^+$ /MeOH, 84%.

*Notes and discussion.* In this method, stable thiophenyl glycoside donors are employed, and NIS is used to effect both tethering and glycosylation of the substrate. Moreover, by using dichloroethane as solvent, it is possible to perform both of these reactions in one-pot.

*Materials.*

Manno enol ether <b>70</b> [59] (138 mg, 0.24 mmol)	treat as toxic
Acceptor <b>71</b> (186 mg, 0.72 mmol)	harmful
4 Å molecular sieves (200 mg)	irritant
<i>N</i> -Iodosuccinimide (161 mg, 0.72 mmol and 107 mg, 0.48 mmol)	harmful
2,6-Di- <i>tert</i> -butyl-4-methyl pyridine (147 mg, 0.72 mmol)	harmful
1,2-Dichloroethane	toxic, flammable
Dichloromethane	harmful
Celite <sup>®</sup>	harmful
10% aq. sodium thiosulfate (25 ml)	harmful
MgSO <sub>4</sub>	treat as toxic
Dowex H <sup>+</sup> ion exchange resin	corrosive
Dichloromethane/ether for chromatography	flammable, harmful

*Equipment.*

Round bottomed flasks (2 × 25 ml) with rubber septa and magnetic stirrer bars  
Magnetic stirrer  
Source of dry argon (or nitrogen) gas  
Reaction dewar and dry ice  
Syringe and needle  
Sinter funnel  
Separating funnel and conical flasks  
Rotary evaporator  
Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

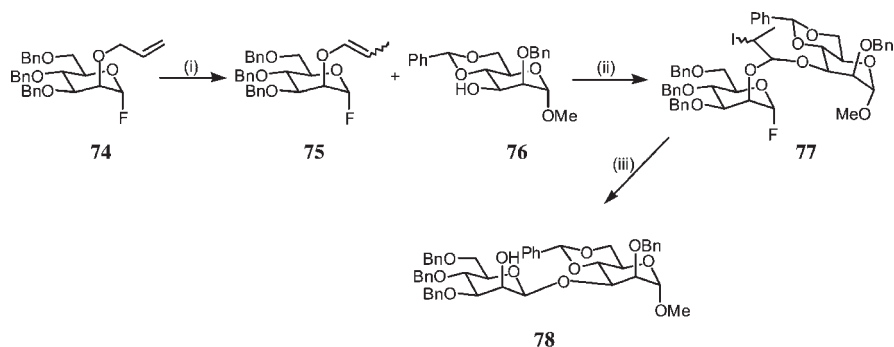
Equip a flame dried round bottomed flask with a magnetic stirrer bar, *manno* enol ether **70** (138 mg, 0.24 mmol), acceptor **71** (186 mg, 0.72 mmol), 4 Å molecular sieves (200 mg) and then purge with argon gas. Add anhydrous 1,2-dichloroethane



(3 ml) and stir the resulting reaction mixture at  $-40\text{ }^{\circ}\text{C}$  under argon. After 10 min add *N*-iodosuccinimide (161 mg, 0.72 mmol), in a solution of 1,2-dichloroethane (1 ml), to the reaction flask and continue stirring at  $-40\text{ }^{\circ}\text{C}$  under argon. After 15 min add further *N*-iodosuccinimide (107 mg, 0.48 mmol) and 2,6-di-*tert*-butyl-methyl pyridine (147 mg, 0.72 mmol) in a solution of 1,2-dichloroethane (4 ml) to the reaction flask and stir at room temperature under argon. After 48 h. add dichloromethane (50 ml) and filter through a Celite<sup>®</sup> pad. Wash the filtrate with 10% aq. sodium thiosulfate (25 ml), dry ( $\text{MgSO}_4$ ), filter and concentrate *in vacuo*. Dissolve the resulting residue in methanol (5 ml), add acidic ion exchange resin (200 mg) and stir at room temperature. After 18 h, filter the reaction mixture through a Celite<sup>®</sup> pad and concentrate the filtrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a dichloromethane/ether (2:1) solvent system to give disaccharide **73** as a colourless syrup (138 mg, 84%);  $[\alpha]_{\text{D}}^{24} = -53$  (*c* 1.3,  $\text{CHCl}_3$ );  $\nu_{\text{max}}$  (thin film) 3584 (br, OH),  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 1.32, 1.34, 1.44, 1.54 (12H,  $4 \times \text{s}$ ,  $4 \times \text{CH}_3$ ), 2.57 (1H, s, OH), 3.42 (1H, ddd,  $J = 9.7, 4.5, 2.2$  Hz, H-5'), 3.56 (1H, dd,  $J = 3.0, 9.1$  Hz, H-3'), 3.70–3.78 (3H, m, H-6a, H-6', H-6a'), 3.92 (1H, a-t,  $J = 9.4$  Hz, H-4'), 4.02–4.05 (1H, m, H-5), 4.14 (1H, dd,  $J = 2.9, 11.2$  Hz, H-6), 4.21–4.23 (2H, m, H-2', H-4), 4.33 (1H, dd,  $J = 2.4, 5.0$  Hz, H-2), 4.50–4.56 (3H, m, H-1',  $\text{PhCH}_2$ ), 4.59–4.67 (3H, m,  $\text{PhCH}_2$ , H-3), 4.80 (1H, d,  $J = 11.9$  Hz,  $\text{PhCH}_2$ ), 4.91 (1H, d,  $J = 10.8$  Hz,  $\text{PhCH}_2$ ), 5.55 (1H, d,  $J = 5.0$  Hz, H-1), 7.18–7.39 (15H, m, ArH);  $^{13}\text{C}$  NMR (50.3 MHz,  $\text{CDCl}_3$ ) 24.3, 24.9, 25.9, 26.1 ( $4 \times \text{CH}_3$ ,  $\text{CH}_3$ ), 69.0, 69.1, 71.1, 75.1 ( $4 \times \text{CH}_2$ ,  $3 \times \text{PhCH}_2$ , C-6, C-6'), 67.7, 67.9, 70.3, 70.7, 71.4, 74.1, 75.2, 81.2 ( $8 \times \text{CH}$ , C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 96.3 (CH, C-1), 100.2 (CH, d,  $J_{\text{C-1',H-1'}} = 157.8$  Hz, C-1'), 108.7, 109.4 ( $2 \times \text{C}$ ,  $2 \times \text{C}(\text{CH}_3)_2$ ), 127.6, 127.7, 127.8, 127.9, 127.9, 128.0, 128.3, 128.4 ( $2 \times \text{CH}$ , ArCH), 137.8, 138.2, 138.8 ( $3 \times \text{C}$ , ArC);  $m/z$  (APCI<sup>+</sup>) 715.6 ( $\text{M} + \text{Na}^+$ , 15%), (HRMS calculated for  $\text{C}_{39}\text{H}_{52}\text{NO}_{11}$  ( $\text{M} + \text{NH}_4^+$ ) 710.3540. Found 710.3552) analysis calculated for  $\text{C}_{39}\text{H}_{48}\text{O}_{11}$ : C, 67.61; H, 7.35. Found: C, 67.10; H, 7.35%.

This methodology proved to be problematic when applied to the tethering and glycosylation of secondary carbohydrate acceptors. However, Fairbanks and co-workers overcame this problem by introducing the 2-*O*-allyl protecting group as a means for tethering an aglycon to the glycosyl donor. Initial studies focused on the use of thioglycosyl donors [60], however, significant improvements in results were observed with the use of fluoroglycosyl donors [61]. The use of glycosyl fluorides is advantageous because they can withstand longer reaction times during the NIS mediated tethering step.

Before tethering can be attempted, the 2-*O*-allyl group must first be isomerised to a vinyl ether. Isomerisation was accomplished by treating glycosyl fluoride **74** with Wilkinson's catalyst and *n*-butyl lithium, which afforded vinyl ether **75** in an excellent yield of 96%. It is important to note that the Wilkinson's catalyst mediated isomerisation is very efficient when compared to the previously used and capricious Tebbe methylenation methodology. Subsequent NIS-mediated tethering of secondary acceptor **76** to vinyl ether **75**



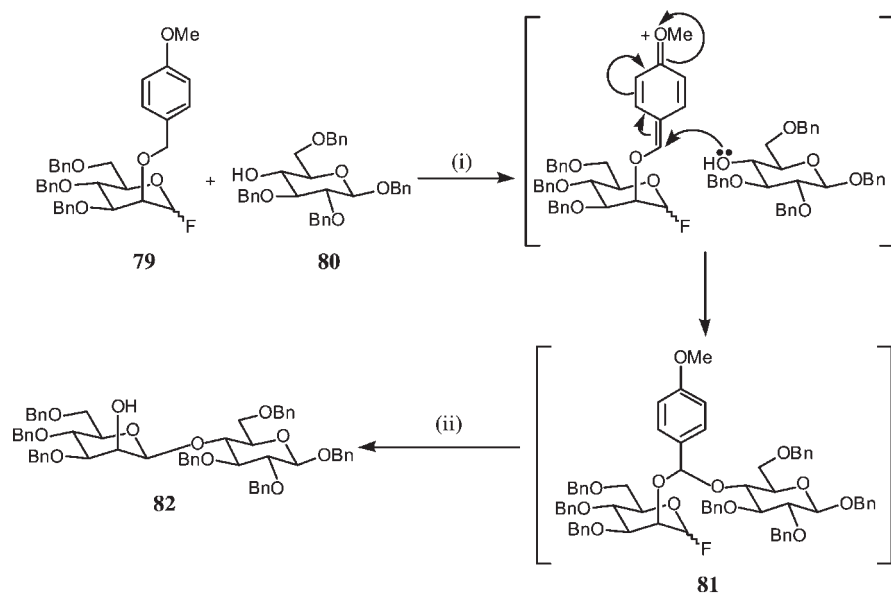
**Scheme 8.17** (i) Wilkinson's catalyst, BuLi, THF, 70 °C, 96%; (ii) NIS, DCE, -40 °C, 83%; (iii) SnCl<sub>2</sub>, DTBMP, DCE, 50 °C, then TFA, H<sub>2</sub>O, 55%.

to afford mixed acetal **77** was achieved in an 83% yield. Treatment of mixed acetal **77** with tin (II) chloride and DTBMP in dichloroethane, followed by the addition of trifluoroacetic acid resulted in IAD to afford  $\beta$ -mannoside **78** in a 55% yield (Scheme 8.17).

The most efficient IAD method to date, developed by Ogawa and co-workers, employs a *para*-methoxybenzyl ether moiety as a temporary tether unit [62]. PMB ethers are a common protecting group in oligosaccharide synthesis and are cleaved by treatment with oxidising agents, such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and cerium ammonium nitrate (CAN). Ogawa and co-workers realised that if a glycosyl acceptor is present during the oxidation of the PMB group it could be trapped by the oxonium ion intermediate thus forming an acetal tether. This was indeed the case, oxidation of the C-2 PMB protected mannosyl fluoride donor **79** with DDQ in the presence of the aglycon **80** resulted in the formation of the tethered acetal intermediate **81**, which was not isolated. Subsequent IAD promoted by AgOTf and SnCl<sub>2</sub> in the presence of the hindered base DTBMP, exclusively afforded the  $\beta$ -1,4-mannoside **82** in a very good yield of 52% (Scheme 8.18)

Encouraged by the use of the PMB-assisted strategy for  $\beta$ -mannosylation, Ogawa and co-workers successfully employed the methodology within the challenging synthesis of the core pentasaccharide of asparagine-linked glycoproteins [63–65]. In this case, glycosylation was accomplished by activating a thiomethyl mannoside donor with methyl triflate.

This methodology was optimised further by protecting the C-4 and C-6 hydroxyl groups with a cyclohexylidene acetal [66]. It was envisaged that the rigid chair conformation of the cyclohexylidene protected thiomethyl mannoside donor **83** would favour an S<sub>N</sub>2 type pathway during the IAD reaction. This in turn would suppress any side reactions occurring from the formation of an anomeric carbonium ion.



**Scheme 8.18** (i) DDQ, DCM, 4 Å mol. sieves, 0 °C to rt; (ii) AgOTf, SnCl<sub>2</sub>, DTBMP, 4 Å mol. sieves, 0 °C to rt, 52%.

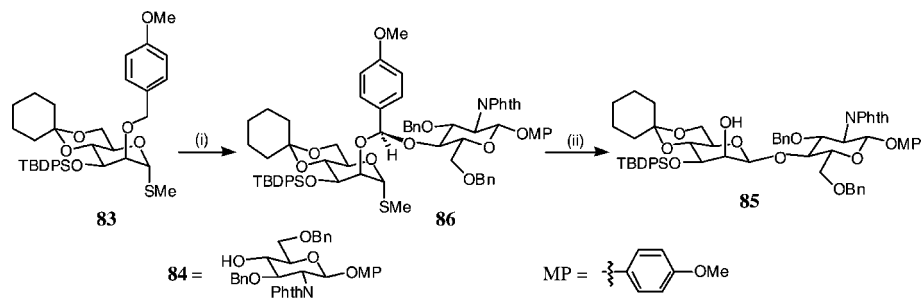
### Method 7

*p*-Methoxybenzylidene assisted intramolecular aglycon delivery [67].

**Notes and discussion.** The PMB-assisted IAD reaction between the cyclohexylidene protected thiomethyl mannoside donor **83** and the very unreactive glucosamine derived acceptor **84** affords exclusive entry to the  $\beta$ -(1,4)-mannoside **85** in an excellent yield of 83%. Advantages of this methodology include: (i) the possibility of using various methods for the introduction of the *O*-*p*-methoxybenzyl group; (ii) the use of mild and near neutral conditions for the formation of the mixed acetal.

### Materials.

Donor <b>83</b> [66] (120.8 mg, 0.186 mmol)	treat as toxic
Acceptor <b>84</b> [66] (81.6 mg, 0.137 mmol)	treat as toxic
2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (48 mg, 0.21 mmol)	harmful
4 Å molecular sieves (200 mg)	irritant
Ascorbic acid (70 mg)	harmful
Citric acid (130 mg)	harmful
Sodium hydroxide (90 mg)	corrosive



(i) **84**, DDQ, 4 Å mol. sieves, DCM, rt; (ii) MeOTf, DTBMP, 4 Å mol. sieves, DCE, 45 °C, 83%.

Methyl triflate (1 M solution in $\text{CCl}_4$ , 0.48 ml, 0.48 mmol)	toxic, corrosive
2,6-Di- <i>tert</i> -butyl-4-methyl pyridine (DTBMP) (98 mg, 0.48 mmol)	harmful
Dichloromethane (4 ml)	harmful
1,2-Dichloroethane (8 ml)	toxic, flammable
Triethylamine	corrosive, flammable
Saturated aq. $\text{NaHCO}_3$	assume toxic
Ethyl acetate	harmful, flammable
$\text{MgSO}_4$	assume toxic
Brine solution	assume toxic
Celite <sup>®</sup>	harmful
Hexane/ethyl acetate for chromatography	flammable, toxic

### *Equipment.*

Round bottomed flasks (1  $\times$  10 ml, 2  $\times$  25 ml) with rubber septa and magnetic stirrer bars  
 Magnetic stirrer/hot plate and oil bath  
 Source of dry argon (or nitrogen) gas  
 Syringe and needle  
 Sinter funnel  
 Separating funnel  
 Rotary evaporator  
 Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

Equip a flame dried round bottomed flask (25 ml) with a magnetic stirrer bar, DDQ (48 mg, 0.21 mmol), 4 Å molecular sieves (200 mg) and then purge with argon gas. Add anhydrous dichloromethane (1 ml) and cool to 0 °C by external cooling. Equip a second flame dried round bottom flask (10 ml) with donor **83** (120.8 mg, 0.186 mmol), acceptor **84** (81.6 mg, 0.137 mmol) and purge with argon gas. Add anhydrous dichloromethane (3 ml), and then add the resulting solution in a dropwise manner to the initial reaction flask. Continue stirring at 0 °C for 15 min and then at room temperature, under argon. After a further 130 min quench the reaction by adding an aqueous solution (10 ml) of ascorbic acid (70 mg), citric acid (130 mg)

and sodium hydroxide (90 mg) to the reaction flask. Dilute the resulting lemon/yellow solution with ethyl acetate (150 ml) and filter through a Celite<sup>®</sup> pad. Wash the filtrate with NaHCO<sub>3</sub> (2 × 20 ml), followed by brine (20 ml), dry the organic phase (MgSO<sub>4</sub>), filter and concentrate *in vacuo* to afford the crude mixed acetal **86** (quantitative). Immediately equip a flame dried round bottomed flask (25 ml) with a magnetic stirrer bar, 4 Å molecular sieves (400 mg), mixed acetal **86**, DTBMP (98 mg, 0.48 mmol) and purge with argon. Add anhydrous 1,2-dichloroethane (8 ml) and stir the resulting reaction mixture at 0 °C by external cooling. Add a solution of methyl triflate in chloroform (1 M, 0.48 ml, 0.48 mmol) to the reaction mixture and continue stirring at 0 °C under argon. After 1 h increase the reaction temperature to 45 °C. After 20 h add triethylamine (~100 µl) to the reaction mixture. Dilute the reaction mixture with ethyl acetate (150 ml) and saturated NaHCO<sub>3</sub> (35 ml) and filter the two-phase solution through a Celite<sup>®</sup> pad. Wash the filtrate with brine (20 ml), dry the organic phase (MgSO<sub>4</sub>), filter and concentrate *in vacuo*. The resulting crude product can be purified by column chromatography using silica gel and a ethyl acetate/hexane (2:5) solvent system to give β-disaccharide **85** as a colourless syrup (122 mg, 83%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +40.7 (c 1.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.11 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.3–2.0 (10H, m, C<sub>6</sub>H<sub>10</sub>), 2.61 (1H, bs, OH), 2.87 (1H, dd, *J* = 10.0, 10.5 Hz, H-5<sub>man</sub>), 3.38–3.43 (2H, m), 3.52–3.64 (3H, m), 3.68 (1H, m), 3.68 (3H, s, OCH<sub>3</sub>), 3.76 (1H, dd, *J* = 10.7, 5.4 Hz), 3.98 (1H, t, *J* = 9.3 Hz), 4.35 (1H, d, *J* = 3.9 Hz, H-2<sub>man</sub>), 4.03 (1H, m), 4.36 (1H, s, H-1), 4.25, 4.45, 4.56, 4.82 (each 1H, d, *J* = 12.0 Hz, PhCH<sub>2</sub>), 5.55 (1H, d, *J* = 8.3 Hz, H-1<sub>GN</sub>), 6.60–7.90 (28H, m, ArH); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) 19.2 (C(CH<sub>3</sub>)<sub>3</sub>), 22.3 (CH<sub>2</sub>), 22.6 (CH<sub>2</sub>), 25.6 (CH<sub>2</sub>), 26.8 (C(CH<sub>3</sub>)<sub>3</sub>), 27.7 (CH<sub>2</sub>), 37.9, (CH<sub>2</sub>), 55.5 (CH), 60.3 (CH<sub>2</sub>), 61.2 (CH<sub>2</sub>), 67.7 (CH), 67.9 (CH<sub>2</sub>), 69.8 (CH), 71.3 (CH), 73.1 (CH), 73.4 (CH<sub>2</sub>), 74.6 (CH), 78.7 (CH), 97.7 (CH, *J*<sub>C-1,H-1</sub> = 165 Hz, C-1<sub>GN</sub>), 99.8 (CH, *J*<sub>C-1,H-1</sub> = 159 Hz, C-1<sub>man</sub>), 100.2 (C); analysis calculated for C<sub>63</sub>H<sub>69</sub>NO<sub>13</sub>Si: C, 70.30; H, 6.46; N 1.30. Found: C, 70.06; H, 6.54; N, 1.31%.

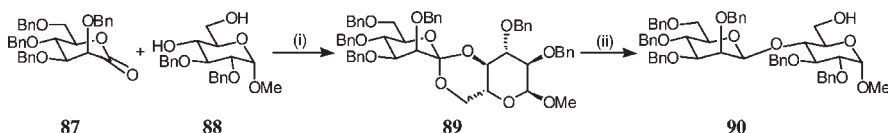
### 8.3.7 Reductive cleavage of cyclic orthoesters

The final method to be discussed in this chapter involves a novel glycosylation strategy inspired by the regiospecific acetal ring opening methodologies of Lipták [68] and Garegg [69]. Ikegami and co-workers have developed a method whereby a cyclic anomeric orthoester is reductively cleaved to generate a β-mannoside. The first step is orthoester formation. This is efficiently achieved by treating sugar lactone **87** and diol **88** with TMSOTf and methoxytrimethylsilane to afford orthoester **89** in a 77% yield [70]. The configuration of the spiro centre of orthoester **89** was confirmed by X-ray crystallography. The subsequent reductive ring opening of orthoester **89** with LiAlH<sub>4</sub> and AlCl<sub>3</sub> proceeded regio- and stereospecifically to afford the β-(1,4)-mannoside **90** in an excellent yield of 98% (Method 8) [70, 71]. By changing the reducing agents to sodium cyanoborohydride

and  $\text{AlCl}_3$  the corresponding  $\beta$ -(1,6)-mannoside can be obtained. The resulting disaccharides from this methodology all contain a free hydroxyl group and could therefore be used as glycosyl acceptors for the synthesis of branched oligosaccharides.

### Method 8

Synthesis of a Man- $\beta$ -(1,4)-Man disaccharide via a spiro orthoester [70, 71].



(i) TMSOMe, TMSOTf,  $\text{PhCH}_3$ , rt, 77%; (ii)  $\text{LiAlH}_4$ ,  $\text{AlCl}_3$ ,  $\text{Et}_2\text{O}/\text{DCM}$  (1:1), rt, 98% over two steps.

**Notes and discussion.** Sugar orthoesters can be efficiently formed by using methoxy silane in the first step of this synthesis, but not *sec*- or *tert*-alkoxy silanes. In order to obtain high yields, the MeOH and hexamethyldisiloxane produced during the reaction must be removed under reduced pressure during the course of the reaction. A range of reducing agents have been considered for effecting conversion of **89** to **90**, but best results were obtained using  $\text{LiAlH}_4/\text{AlCl}_3$ .

### Materials.

Lactone <b>87</b> [72] (400 mg, 0.74 mmol)	treat as toxic
Diol <b>88</b> [73] (277 mg, 0.74 mmol)	treat as toxic
Trimethylsilyl triflate ( $2 \times 7 \mu\text{l}$ , 5 mol%)	corrosive
Methoxy trimethylsilane ( $2 \times 1 \text{ ml}$ , $2 \times 7.4 \text{ mmol}$ )	corrosive
Toluene	flammable and harmful
Lithium aluminium hydride (4 mg, 0.05 mmol)	avoid contact with water
Aluminium chloride (13 mg, 0.1 mmol)	corrosive
Dichloromethane	harmful
Diethyl ether	flammable, harmful
Saturated potassium sodium tartrate	assume toxic
Brine	assume toxic

MgSO<sub>4</sub>

assume toxic

Hexane/ether for chromatography

flammable, toxic

*Equipment.*

Round bottomed flasks (1 × 10 ml, 1 × 25 ml) with rubber septa and magnetic stirrer bars

Magnetic stirrer

Source of dry argon (or nitrogen) gas

Syringe and needle

Sintra funnel

Separating funnel

Rotary evaporator

Column chromatography equipment

*Experimental procedure.* Safety glasses should be worn during the experiment and all manipulations should be performed in a fume cupboard.

*Step (i).* Equip a flame dried round bottomed flask with a magnetic stirrer bar, lactone **87** (400 mg, 0.74 mmol), diol **88** (277 mg, 0.74 mmol) and then purge with argon. Add anhydrous toluene (5 ml), followed by methoxy trimethylsilane (1.0 ml, 7.4 mmol) and trimethylsilyl triflate (7 µl, 5 mol%) and stir at room temperature under argon. After 1 h concentrate the reaction mixture *in vacuo*. Purge the reaction flask with argon, add further anhydrous toluene (5 ml) followed by methoxy trimethylsilane (1.0 ml, 7.4 mmol) and trimethylsilyl triflate (7 µl, 5 mol%) and stir at room temperature under argon. After 30 min concentrate the reaction mixture *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a ether/hexane (1:3) solvent system to give orthoester **89** as a white crystalline solid (77%); mp 107.5–108.5 °C;  $[\alpha]_D^{21} = -6.9$  (c 1.0, CHCl<sub>3</sub>);  $\nu_{\max}$  (KBr) 3374, 2930, 2587, 2361, 2339, 1711, 1460, 1377 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 3.39 (3H, s, OCH<sub>3</sub>), 3.52 (1H, dd, *J* = 3.7, 9.2 Hz, H-2), 3.60 (1H, ddd, *J* = 9.6, 6.1, 2.0 Hz, H-5), 3.71–3.82 (5H, m, H-5, H-6, H-6', H-6'), 3.84 (1H, d, *J* = 3.1 Hz, H-2'), 3.93 (1H, t, *J* = 9.2 Hz, H-3), 3.94 (1H, dd, *J* = 3.1, 9.6 Hz, H-3'), 4.02 (1H, t, *J* = 9.6 Hz, H-4'), 4.17 (1H, t, *J* = 9.2 Hz, H-4), 4.52 (1H, d, *J* = 11.3 Hz, PhCH<sub>2</sub>), 4.52 (1H, d, *J* = 11.3 Hz, PhCH<sub>2</sub>), 4.54 (1H, d, *J* = 3.7 Hz, H-1), 4.56 (1H, d, *J* = 11.9 Hz, PhCH<sub>2</sub>), 4.61 (1H, d, *J* = 12.2 Hz, PhCH<sub>2</sub>), 4.64 (1H, d, *J* = 12.2 Hz, PhCH<sub>2</sub>), 4.72 (1H, d, *J* = 12.5 Hz, PhCH<sub>2</sub>), 4.72 (1H, d, *J* = 12.2 Hz, PhCH<sub>2</sub>), 4.77 (1H, d, *J* = 11.9 Hz, PhCH<sub>2</sub>), 4.82 (1H, d, *J* = 11.9 Hz, PhCH<sub>2</sub>), 4.87 (1H, d, *J* = 11.6 Hz, PhCH<sub>2</sub>), 4.90 (1H, d, *J* = 11.3 Hz, PhCH<sub>2</sub>), 4.96 (1H, d, *J* = 11.9 Hz, PhCH<sub>2</sub>), 7.13–7.36 (30H, m, ArH); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) 55.3 (OCH<sub>3</sub>), 61.1, 62.2, 68.9, 71.7, 73.2, 73.9, 74.1, 74.3, 74.5, 75.1, 75.9, 78.8, 79.1, 80.8, 99.3 (C-1), 110.7 (C-1'), 127.2, 127.3, 127.4, 127.5, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 138.2, 138.5, 138.6; *m/z* (FAB) 933 (M + K<sup>+</sup>), 917 (M + Na<sup>+</sup>), 895 (M + H<sup>+</sup>), 826 (M – Bn + Na<sup>+</sup>); HRMS calculated for C<sub>55</sub>H<sub>58</sub>O<sub>11</sub>Na 917.3877. Found 917.3892.



*Step (ii).* Equip a flame dried round bottomed flask (10 ml) with a magnetic stirrer bar, orthoester **89** (45 mg) and then purge with argon. Add anhydrous diethyl ether (0.5 ml) and anhydrous dichloromethane (0.5 ml), followed by lithium aluminium hydride (4 mg, 0.1 mmol). Add aluminium chloride (13 mg) in a solution of anhydrous ether (0.5 ml) dropwise to the reaction mixture and stir at room temperature under argon. After 1 h dilute the reaction mixture with anhydrous ether (5 ml) and then cautiously add saturated potassium sodium tartrate solution (5 ml). Vigorously stir the resulting mixture for 30 min and then carefully separate the ether layer. Extract the aqueous layer with ether (2  $\times$  50 ml) and dichloromethane (50 ml). Combine the organic extracts, dry ( $\text{MgSO}_4$ ), filter and concentrate *in vacuo*. Purify the resulting crude product by column chromatography using silica gel and a ether/hexane (2:1) solvent system to give disaccharide **90** as a colourless syrup (98%);  $[\alpha]_D^{24} = -14.3$  (*c* 1.1,  $\text{CHCl}_3$ );  $\nu_{\text{max}}$  (thin film) 3463, 3031, 2924, 1605, 1497, 1455, 1364  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 2.20 (1H, bs, OH), 3.35 (3H, s,  $\text{OCH}_3$ ), 3.39 (1H, dd,  $J = 3.7, 9.5$  Hz, H-2), 3.43 (1H, ddd,  $J = 2.7, 6.1, 9.2$  Hz), 3.48 (1H, dd,  $J = 2.9, 9.0$  Hz), 3.56–3.65 (3H, m), 3.69–3.78 (2H, m), 3.81–3.95 (4H, m), 4.42 (1H, d,  $J = 12.2$  Hz,  $\text{PhCH}_2$ ), 4.47 (1H, d,  $J = 12.2$  Hz,  $\text{PhCH}_2$ ), 5.51 (1H, d,  $J = 10.7$  Hz,  $\text{PhCH}_2$ ), 4.52 (1H, d,  $J = 3.4$  Hz, H-1), 4.53 (1H, d,  $J = 12.9$ ,  $\text{PhCH}_2$ ), 4.59 (1H, d,  $J = 11.9$  Hz,  $\text{PhCH}_2$ ), 4.63 (1H, bs,  $J = <0.5$  Hz, H-1'), 4.76 (1H, d,  $J = 12.2$  Hz,  $\text{PhCH}_2$ ), 4.80 (1H, d,  $J = 11.6$  Hz,  $\text{PhCH}_2$ ), 4.81 (1H, d,  $J = 11.6$  Hz,  $\text{PhCH}_2$ ), 4.84 (1H, d,  $J = 12.2$  Hz,  $\text{PhCH}_2$ ), 5.03 (1H, d,  $J = 11.0$  Hz,  $\text{PhCH}_2$ ), 7.15–7.40 (30H, m, ArH);  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ ) 55.2 ( $\text{OCH}_3$ ), 62.0, 69.7, 70.7, 71.9, 73.3, 73.5, 73.8, 74.6, 74.8, 75.0, 75.2, 75.9, 76.7, 79.6, 80.0, 82.1, 98.2 (C-1), 100.1 (C-1'), 127.2, 127.3, 127.6, 127.6, 127.8, 128.0, 128.1, 128.1, 128.2, 128.3, 128.3, 128.4, 138.1, 138.2, 138.3, 138.6, 138.8, 139.4;  $m/z$  (FAB) 935 ( $\text{M} + \text{K}^+$ ), 919 ( $\text{M} + \text{Na}^+$ ), 828 ( $\text{M} - \text{Bn} + \text{Na}^+$ ); HRMS calculated for  $\text{C}_{55}\text{H}_{60}\text{O}_{11}\text{Na}$  919.4034. Found 919.4015.

## 8.4 CONCLUSION

This chapter has illustrated the importance of the  $\beta$ -mannosidic linkage within biological systems, and problems encountered with its synthesis. A number of chemical preparative methods have been compared, but it is obvious that there is no one, ideal method of universal utility. As with all carbohydrate chemistry, a careful assessment of the proposed preparative route must be performed before a specific technique for the incorporation of the  $\beta$ -mannosidic linkage is selected and pursued.

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# Synthesis of Sialic Acid Containing Carbohydrates

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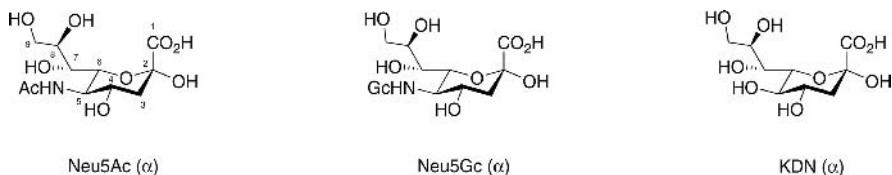
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## 9.1 INTRODUCTION

The sialic acids are now defined as a family of carbohydrates that are derived from 3-deoxy-non-2-ulosonic acid. The major three sialic acids found in nature are *N*-acetyl (Neu5Ac) and *N*-glycolyl (Neu5Gc) derivatives of neuraminic acid (5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid), and 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (KDN) (Figure 9.1). These sialic acids terminate oligosaccharide chains onto lipid and protein scaffolds through  $\alpha$ -(2  $\rightarrow$  3) or  $\alpha$ -(2  $\rightarrow$  6) linkages with Gal, GalNAc or GlcNAc residues and  $\alpha$ -(2  $\rightarrow$  8) linkages with another neuraminic acid residue [1]. The sialylation of carbohydrates in cells is catalyzed by a family of sialyltransferases in the Golgi apparatus and the sialo-descendants thus formed play crucial roles in biological processes such as cell growth, cell differentiation, cell adhesion, cell-mediated immune response and oncogenesis, and also function as receptors for hormones, viruses and bacterial toxins [2]. The recent findings concerning the diverse functions of sialoglycoconjugates have spurred the exploration of sialoglyco-chemistry, in which modern carbohydrate chemistry [3], that could fabricate homogeneous sialoglycoconjugates in large quantities, has opened the gate to sialoglycobiology research at the molecular level. Such studies have provided clear insight into their importance for modulating cell–cell recognition and signaling and also clues for carbohydrate-based drugs [4].

The chemistry of sialic acid was pioneered in the late 1960s and focussed on the preferential formation of the  $\alpha$ -ketosidic linkage. This is a particularly difficult chemical problem since the anomeric centre sterically and electronically disfavours formation of the oxocarbenium ion intermediate in the course of glycosidation.



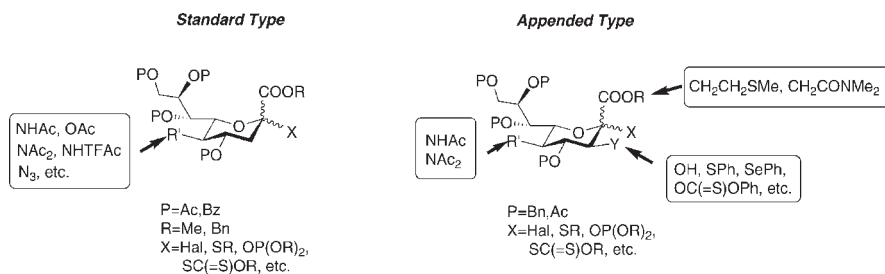
**Figure 9.1** Representative Sialic acids [1b].

Also, no neighbouring substituent is present at C-3 to assist with the formation of the alternative thermodynamically more stable  $\beta$ -glycoside. A wide spectrum of exquisite devices is available for the chemical synthesis of  $\alpha$ -sialosides, and these utilize a variety of sialic acid donors. However, no single sialic acid donor is available that is applicable to every synthetic strategy.

In this chapter, the chemistry of sialic acid is briefly reviewed and some advantages and problems of strategies described for the construction of the  $\alpha$ -ketosidic linkage of sialic acid and complicated sialosides are presented. Experimental details are also provided to allow practical preparation of the chosen targets.

## 9.2 SIALIC ACID DONORS

Sialic acid donors that have been reported to date can be classified into two major types based on the mode of chemical modification. These have been termed standard type and appended type donors (Figure 9.2). All of the donors have a suitable leaving group such as bromide, chloride, alkyl- or arylthio group (SMe, SEt, SPh, etc.) or phosphite group [OP(OEt)<sub>2</sub> and OP(OBn)<sub>2</sub>] at the anomeric C-2 carbon. In the appended type donors an assisting nucleophilic functionality (auxiliary) is mounted at C-3 or C-1 to restrain the nucleophile from attacking the  $\beta$ -face. In contrast, the standard type donors maintain the 3-deoxy structure.



**Figure 9.2** Sialic acid donors.

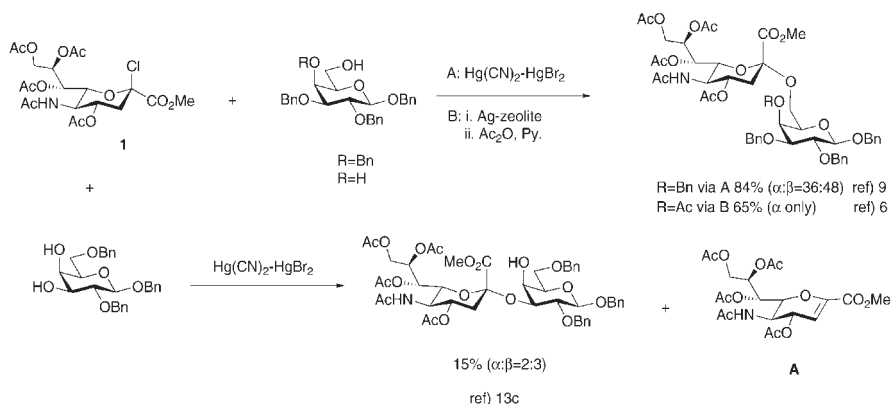
### 9.2.1 Standard type donors

Initial studies to construct the  $\alpha$ -glycoside of *N*-acetyl neuraminic acid (Neu5Ac) employed the 2-chloride donor derivative [5]. It is relatively easy to access most

standard donors of Neu5Ac via three step-reaction sequences from the free Neu5Ac. Due to the characteristic 3-deoxy structure, it is not possible to influence  $\alpha$ -selective glycosidation via neighboring group participation. In addition, standard type donors are prone to form 2,3-dehydro derivatives as a consequence of the electron withdrawing carboxyl group at the anomeric centre. Therefore, the anomeric selectivity and coupling yield are strongly dependent on the reaction conditions, e.g. solvent, promoter system and temperature. An enormous amount of data is available in the literature that provide results of studies within this area [3f–n].

### 2-Halogeno type

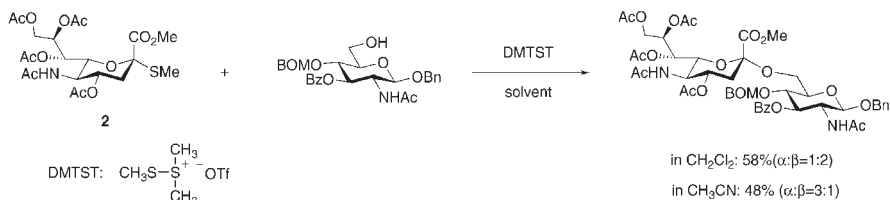
The 2-chloro derivatives **1** [5] have been more widely utilized for  $\alpha$ -sialidation reactions with acceptors, that vary from simple alcohols to trisaccharides, than the corresponding 2-fluoro [6] or bromo [7] derivatives. The 2-Cl derivative is immediately available from the corresponding methyl ester derivative of sialic acid by treatment with AcCl [8] or from the 2-*O*-acetyl derivative of sialic acid by the action of TiCl<sub>4</sub> [9], HCl/AcCl [10] or AcCl/MeOH [11]. It is moderately stable and can be kept for several weeks in a freezer. Activation of the 2-Cl donors can be effected with Ag<sub>2</sub>CO<sub>3</sub> [5, 9, 12], Hg(CN)<sub>2</sub>/HgBr<sub>2</sub> [9, 13] other insoluble silver salts, i.e. silver polymaleate [14], silver salicylate [15] and silver zeolite [16], silver mercaptoethanesulfonate [17], Lewis acids [18] or I<sub>2</sub> [19]. The coupling reactions using the 2-Cl donors tend to exhibit high yields with highly reactive acceptors but low yields with poor acceptors, e.g. those with a hindered hydroxyl group. In the latter case the 2,3-dehydro derivative is formed as a major byproduct and the reactions also exhibit low  $\alpha$ -stereoselectivity in many cases (Scheme 9.1). Therefore the usage of 2-Cl sialic acid derivatives is generally restricted to the preparation of sialosides of simple alcohols [12a, 15d,e, 17, 19], thioglycosides [20] or the corresponding 2,3-dehydro derivatives [8b].



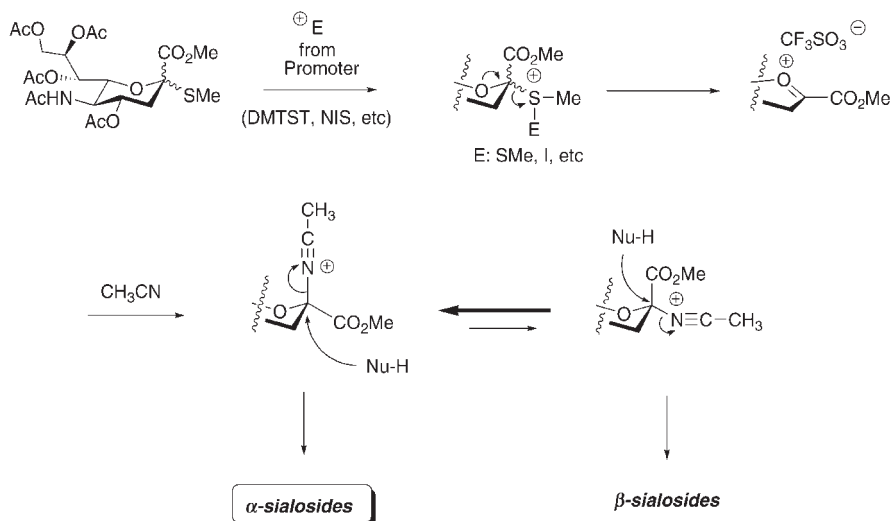
**Scheme 9.1** Coupling reactions using 2-Cl donor.

## 2-Thioglycosides

Thioglycoside derivatives of sialic acids, for example, methyl [21]-, ethyl [22]- and phenyl [22a, 23, 24]-thioglycosides, have emerged as the second generation of standard type sialic acid donors. They can be obtained in three steps from Neu5Ac and/or KDN, and exhibit long shelf life stabilities. The methylthioglycoside donor **2** [21a] has proved the excellent versatility of thioglycosides for effecting  $\alpha$ -glycosylation upon activation with dimethyl(methylthio)sulfonium triflate (DMTST) [25]. In particular it is noteworthy that high  $\alpha$ -stereoselectivity was only obtained when the reaction was conducted in acetonitrile (Scheme 9.2) [21a, 26]. This result has been explained via the following proposed reaction mechanism; a thiophilic electrophile ( $^+\text{SMe}$ ) from DMTST reacts with a lone pair on sulfur to afford a cationic sulfonium species, which is an excellent leaving group. The sulfonium intermediate subsequently reacts with nitrogen from the acetonitrile, to preferentially afford the  $\beta$ -nitrilium ion. This then undergoes  $\text{S}_\text{N}2$  nucleophilic substitution with a sugar hydroxyl to predominantly afford the  $\alpha$ -glycosides (Figure 9.3) [27]. Formation of the favored  $\beta$ -nitrilium ion is implied by evidence that the  $\alpha$ -glucopyranosyl nitrilium ion was generated from the corresponding oxocarbenium ion in dry acetonitrile [28].  $\alpha$ -Selective sialylation can also be effected with other thiophilic promoters, such as *N*-iodosuccinimide (NIS)-triflic acid ( $\text{TfOH}$ ) [29–30] or  $\text{TMSOTf}$  [31],  $\text{PhSeOTf}$  [32],  $\text{NBS-Bu}_4\text{NOTf}$  or  $\text{Ph}_2\text{IOTf}$  [33] and  $\text{CH}_3\text{SBr-AgOTf}$  [34], and nitrile solvents such as acetonitrile and propionitrile (Scheme 9.3). In recent examples, the inexpensive phenylthio derivative **5** [22] has been utilized as a surrogate for the methylthio donor [35]. Iodonium promoter systems are usually used for these donors in preference to DMTST and such donors sometimes afford higher yields in glycosidation reactions than methylthio donors (Scheme 9.4) [31, 36]. Utilization of the phenylthio glycoside donor of dimeric, trimeric and tetrameric sialic acid has also accomplished the synthesis of sialo-oligosaccharides in moderate yields [37]. Syntheses of a range of sialo-oligosaccharides, for example, sialyl Lewis X [38], GM1 [39], GT1 $\alpha$  [31], PSGL-1 [40] have been achieved using the thioglycoside donors [41], indicating that they fulfill the stringent criteria for being highly practical sialyl donors.



**Scheme 9.2** Initial experiments for  $\alpha$ -predominant sialylation in acetonitrile.<sup>21a</sup>



**Figure 9.3** Proposed mechanism of nitrilium solvent assisted  $\alpha$ -sialylation.

### 2-Phosphites and xanthates

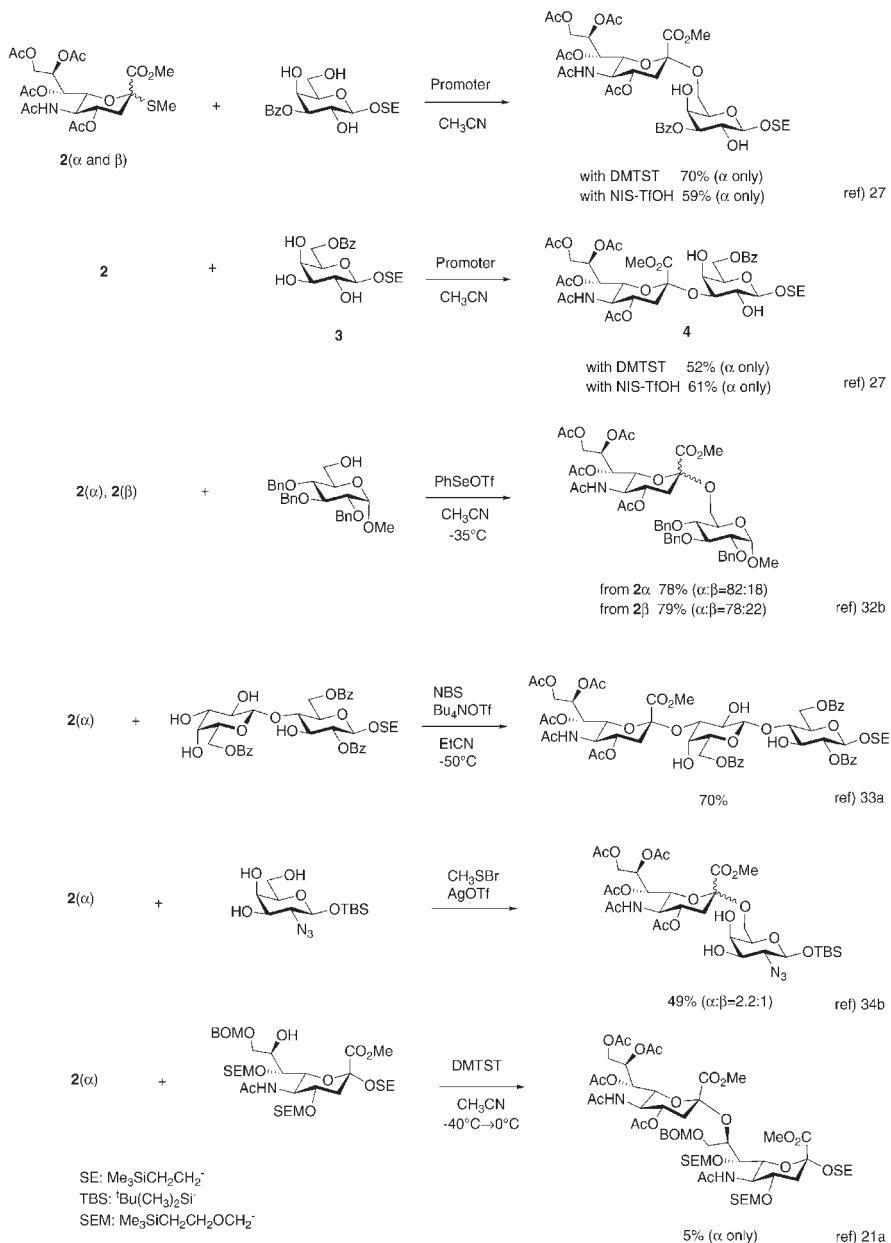
Schmidt and Wong have developed 2-diethyl- and dibenzyl-phosphite derivatives of sialic acid, **7** [42] and **8** [43] (Scheme 9.4), respectively. Nitrile solvents again effect  $\alpha$ -predominant sialylation. The efficacy of diethylphosphite donors as useful alternatives to more traditional donors was exemplified by their incorporation within many linear and convergent syntheses of complicated sialyl oligosaccharides [30d, 44]. The catalytic activation of the phosphite donors by acids [ $\text{TMSOTf}$ ,  $\text{Sn}(\text{OTf})_2$ , etc.] has led to the development of strategies that provide minimal exposure of the substrates to acid, which is useful if acid labile protecting groups are incorporated within the molecules. In addition, activation of the diethylphosphite derivatives in a chemo-selective manner with phosphorodiamidite and  $\text{TfOH}$  has also been demonstrated (Scheme 9.5) [45].

An examination of other xanthate derivatives has also been conducted. Exclusive  $\alpha$ -glycosidation was achieved by exploiting  $\text{CH}_3\text{SBr}-\text{AgOTf}$  as the promoter in acetonitrile (Scheme 9.6) [22b, 46]. Several groups have successfully incorporated 2-xanthate donors within a variety of oligosaccharide syntheses [47].

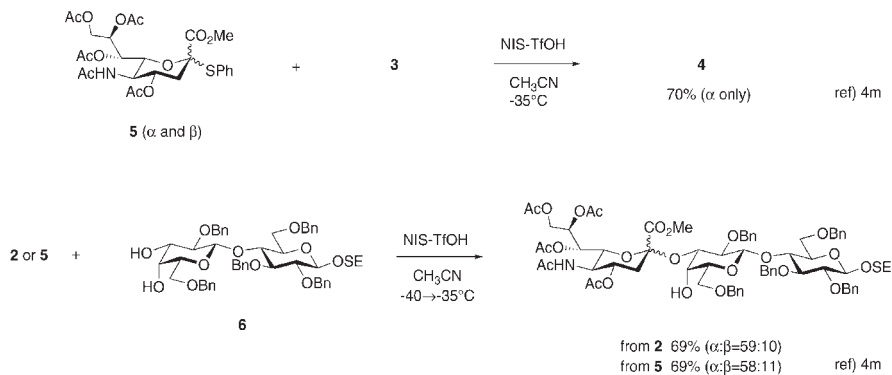
### New entries

A third generation of thioglycoside donors, namely,  $N,N$ -diacetyl [48],  $N$ -trifluoroacetyl (TFAc) [49] and azido [50] derivatives has recently emerged. All of these donors can potentially exhibit higher yields and  $\alpha$ -selective glycosidations than traditional donors (Scheme 9.7a and b). In particular, the coupling of the  $N$ -TFAc donor **11** with the least reactive C-8 hydroxyl of a sialic acid acceptor is unparalleled, affording the disaccharide in 55% yield with the  $\alpha$ -anomer being

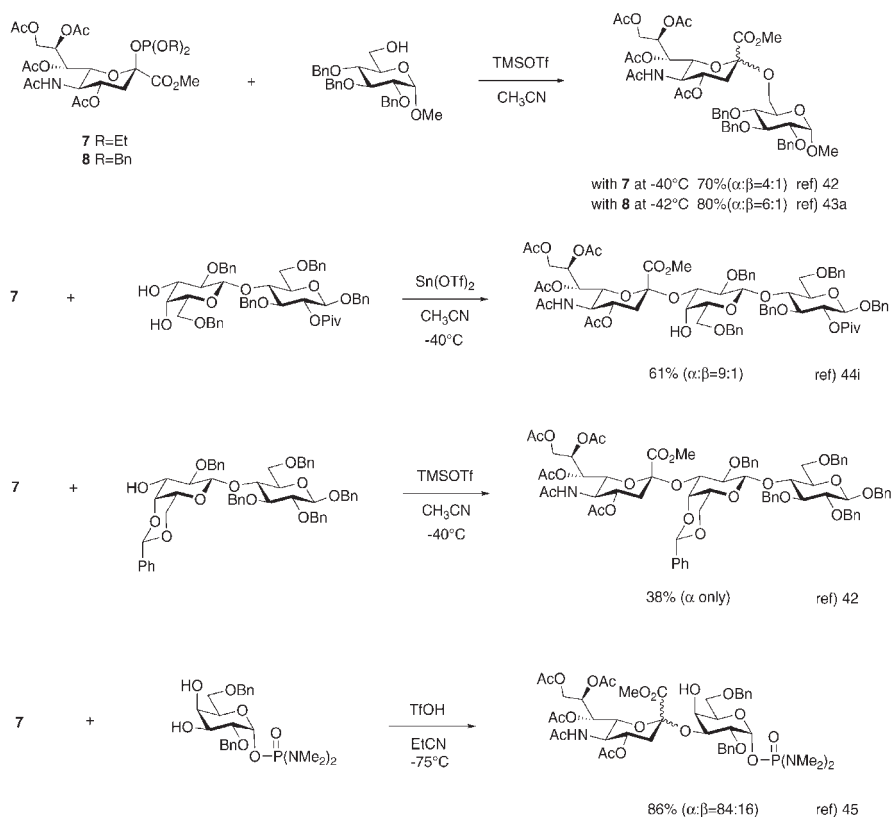


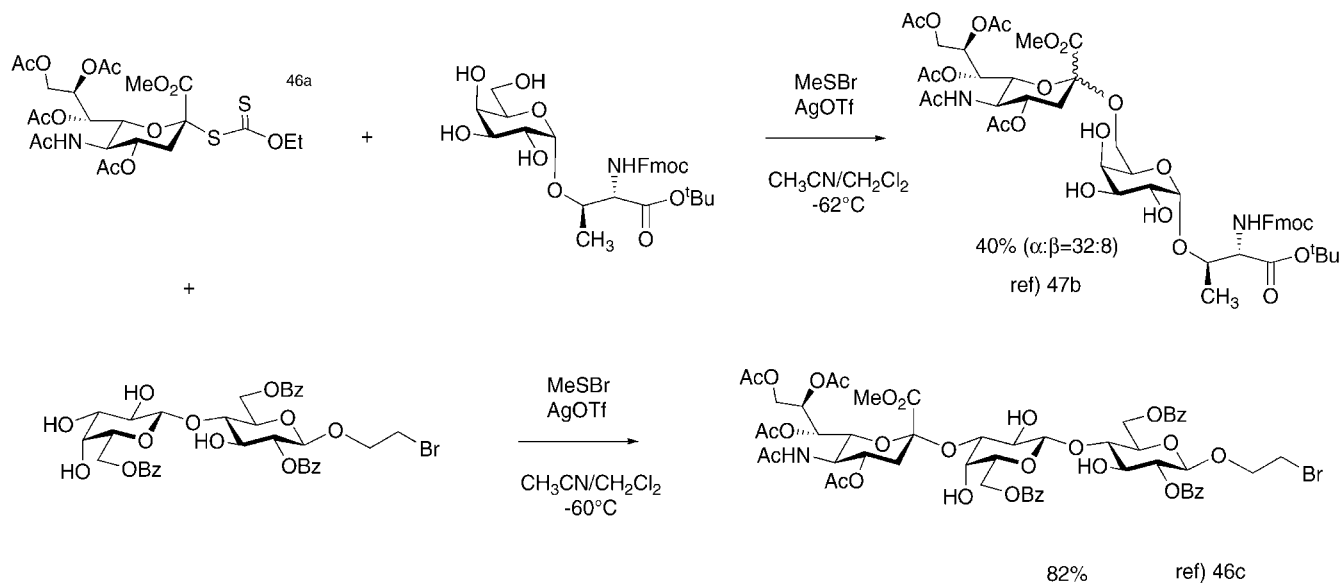


**Scheme 9.3** α-Predominant sialylations using 2-methylthio donor.

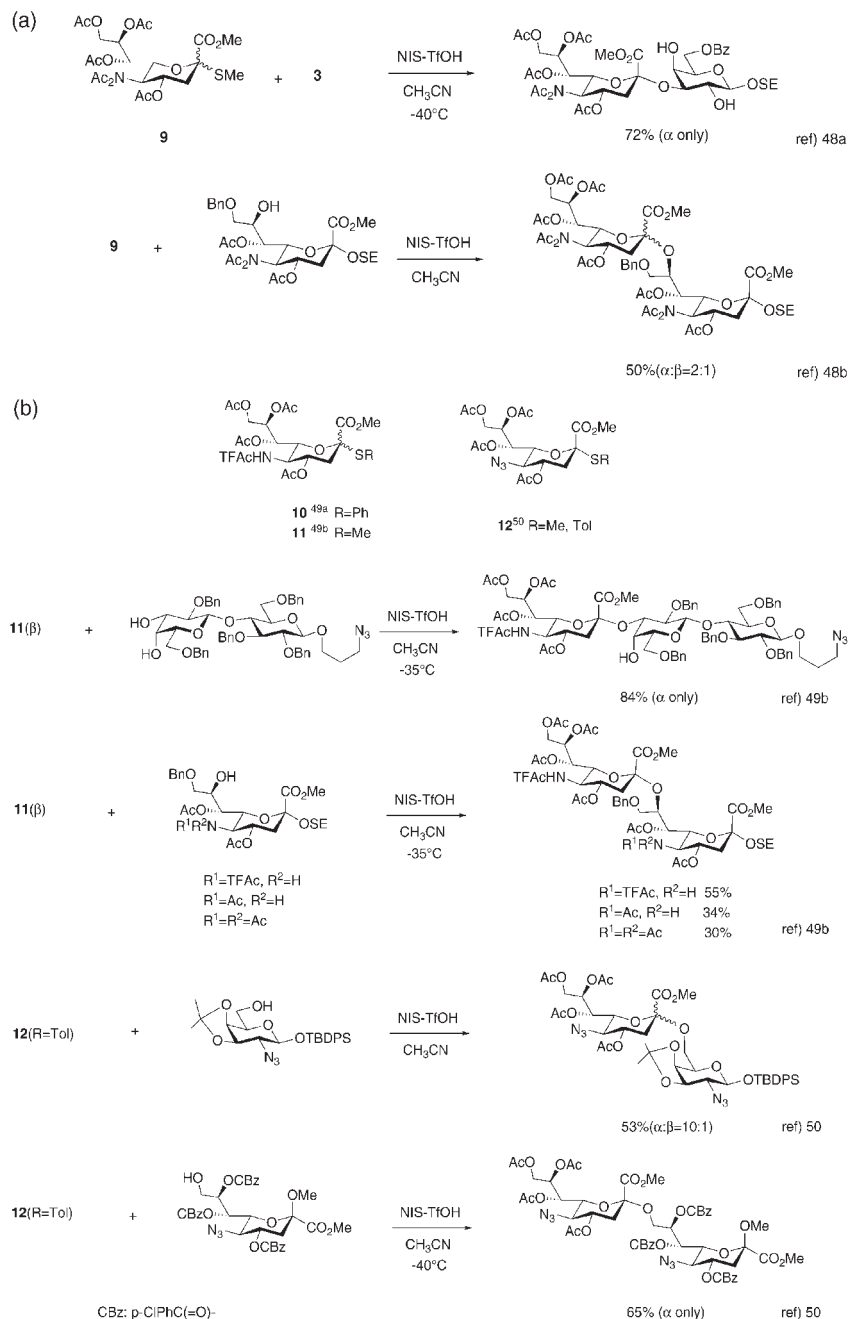


Scheme 9.4

Scheme 9.5 Application of 2-phosphite donors to  $\alpha$ -sialylation.



Scheme 9.6



Scheme 9.7

exclusively formed [49b]. Also, from a strategic point of view, utilization of the *N*-TFAc donor is profitable since it can be pivotally transformed into the corresponding *N*-acetyl, *N*-glycolyl and  $\text{NH}_2$  derivatives following facile protocols. However, it should be noted that it is harder to access the *N*-TFAc donor from Neu5Ac than other standard type donors.

### 9.2.2 Appended type donors

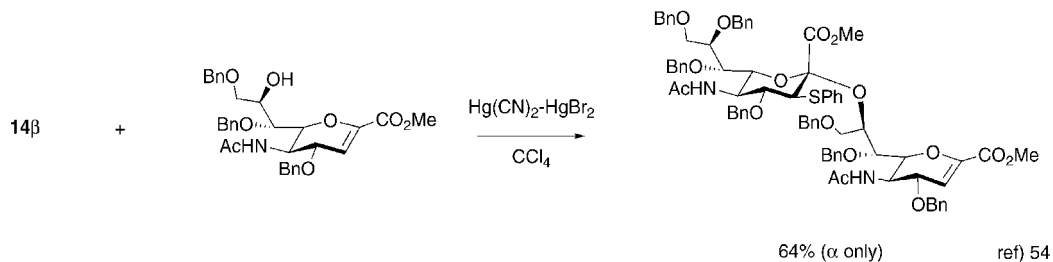
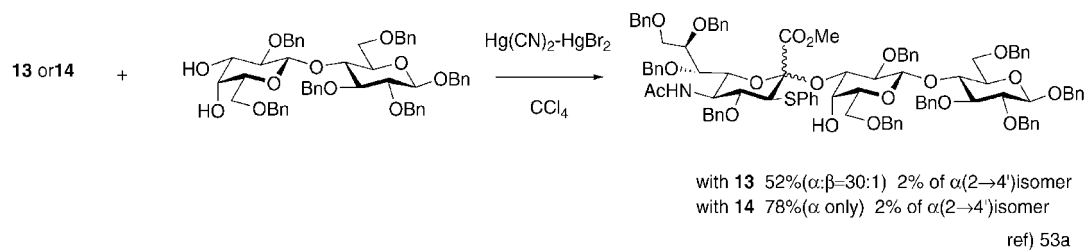
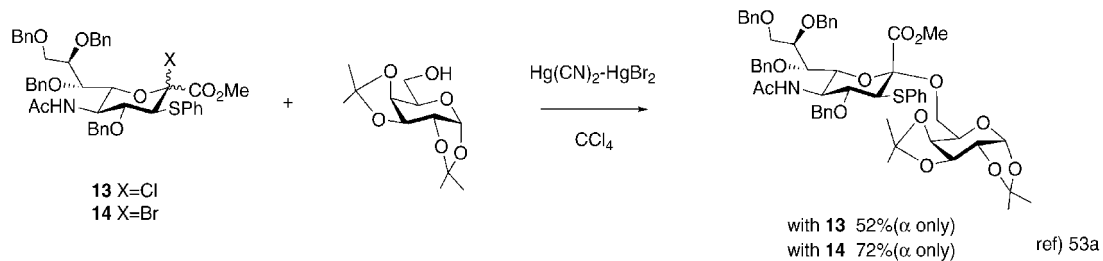
The appended type donors incorporate neighbouring functionalities at C-3 or C-1 in order to direct the  $\alpha$ -selective assembly of sialosides. Therefore,  $\alpha$ -selective sialylation using appended type donors is independent of solvent.

#### *C-3 type*

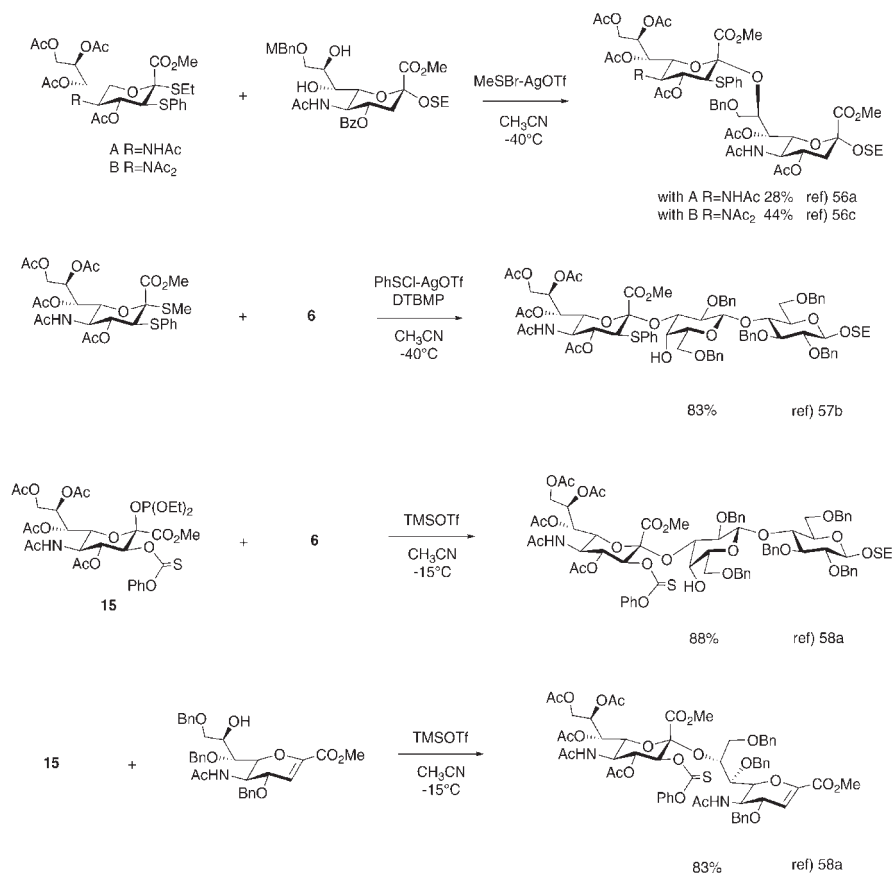
Many equatorial participating auxiliaries have been incorporated at C-3 that are able to nucleophilically participate with the contiguous anomeric carbon during condensation. For example, hydroxyl [51], phenylseleno [52] and phenylthio [53] groups have all been documented. In comparison with standard type donors, the appended type donors offer better  $\alpha$ -selectivity and increased yield, which can be attributed to minimal production of the 2,3-dehydro derivative. Among the appended type donors, the 3-thiophenyl derivative **14** [53], devised by Ito and Ogawa, has proved invaluable in glycosylations, and this donor has even provided excellent results with severely hindered acceptors such as the C-8 hydroxyl acceptor of Neu5Ac derivatives (64%, exclusively  $\alpha$ ) (Scheme 9.8). Indeed, the challenging oligosaccharide, ganglioside  $\text{GD}_3$  which contains the Neu5Ac $\alpha(2 \rightarrow 8)$  Neu5Ac residue was first synthesized by exploiting this 3-thiophenyl donor [54]. It has also proved the donor of choice in polymer-supported synthetic strategies [55]. According to the recent literature, the combined use of a C-3 auxiliary and exploitation of the nitrile effect leads to exclusive  $\alpha$ -sialylation (Scheme 9.9) [56–58]. However, arduous protocols for connection and disconnection of the auxiliaries sometimes detract from the broad utility of the appended type donors.

#### *C-1 type*

Incorporation of an auxiliary at C-1, which is anticipated to show long-range reaction with the  $\beta$ -face of the anomeric carbon (Figure 9.4), was originally examined by Takahashi. The 2-methylthioethyl functionality was incorporated through an ester linkage at C-1 of the 2-methylthio derivative of Neu5Ac [59]. A coupling reaction of the C-1 appended donor with a glycosyl acceptor in dimethylether gave the  $\alpha$ -glycoside in relatively high selectivity but with low yield. In an alternative approach, a C-1 appended donor incorporating an *N,N*-dimethylglycolamide ester, as developed by Gin, afforded  $\alpha$  ( $2 \rightarrow 3$ )sialyl-Gal derivative in 80% yield as a mixture of  $\alpha$ -(60%) and  $\beta$ -(20%) isomers [60] (Scheme 9.10).



Scheme 9.8



Scheme 9.9

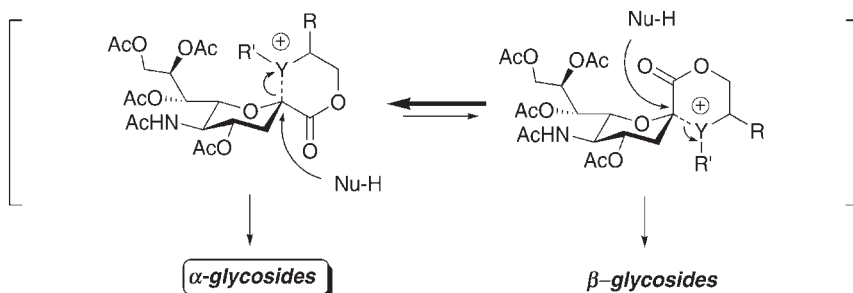
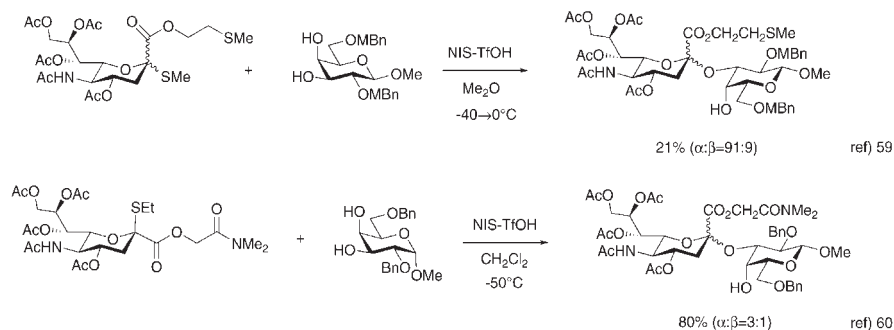


Figure 9.4 Long-range participation of C-1 auxiliary.



Scheme 9.10

### 9.3 SIALYLATION IN OLIGOSACCHARIDE SYNTHESIS

#### 9.3.1 Sialylation with an oligosaccharide acceptor

Several groups have reported linear synthetic approaches to sialyl-oligosaccharides that involves sialylation to large, complex oligosaccharides [18i,j, 30d, 34a,c, 35a, 41c,g,i, j, 44d, 47g]. The feasibility of such sialylations is not predictable due to the dependence of the efficiency of the reaction on the structure of the acceptor. In general, optimum results are obtained when the number of protecting groups within the acceptor is kept to a minimum. In fact, excellent results have been obtained in glycosidations with free diol and triol acceptors (Figure 9.5). It should also be noted that the concomitant production of the  $\beta$ -isomer can sometimes cause fatal problems in syntheses, due to the difficulty of anomer separation by column chromatography.

#### 9.3.2 Sialyl building blocks

Considering the inherent problems associated with the linear synthesis of sialo-oligosaccharides, a convergent approach using sialyl building blocks may often be more suitable. In this context a wide range of sialyl building blocks [20c, 30f, 41k, 44h, 53f] such as sialyl- $\alpha$ -(2  $\rightarrow$  3')Lac [32d, 39, 44a] and lactosamine [30e], sialyl- $\alpha$ -(2  $\rightarrow$  3) [5, 37e, 38, 39, 40, 41a,f,h,l, 61, 62] and  $\alpha$ -(2  $\rightarrow$  6) [41b,e, 46a] Gal derivatives have been utilized for complicated oligosaccharide syntheses. Since a number of biologically relevant glycan chains contain the sialyl  $\alpha$ -(2  $\rightarrow$  3)Gal moiety, incorporation of the corresponding building blocks within the syntheses of a range of oligosaccharides is highly conspicuous in the literature (Figure 9.6).



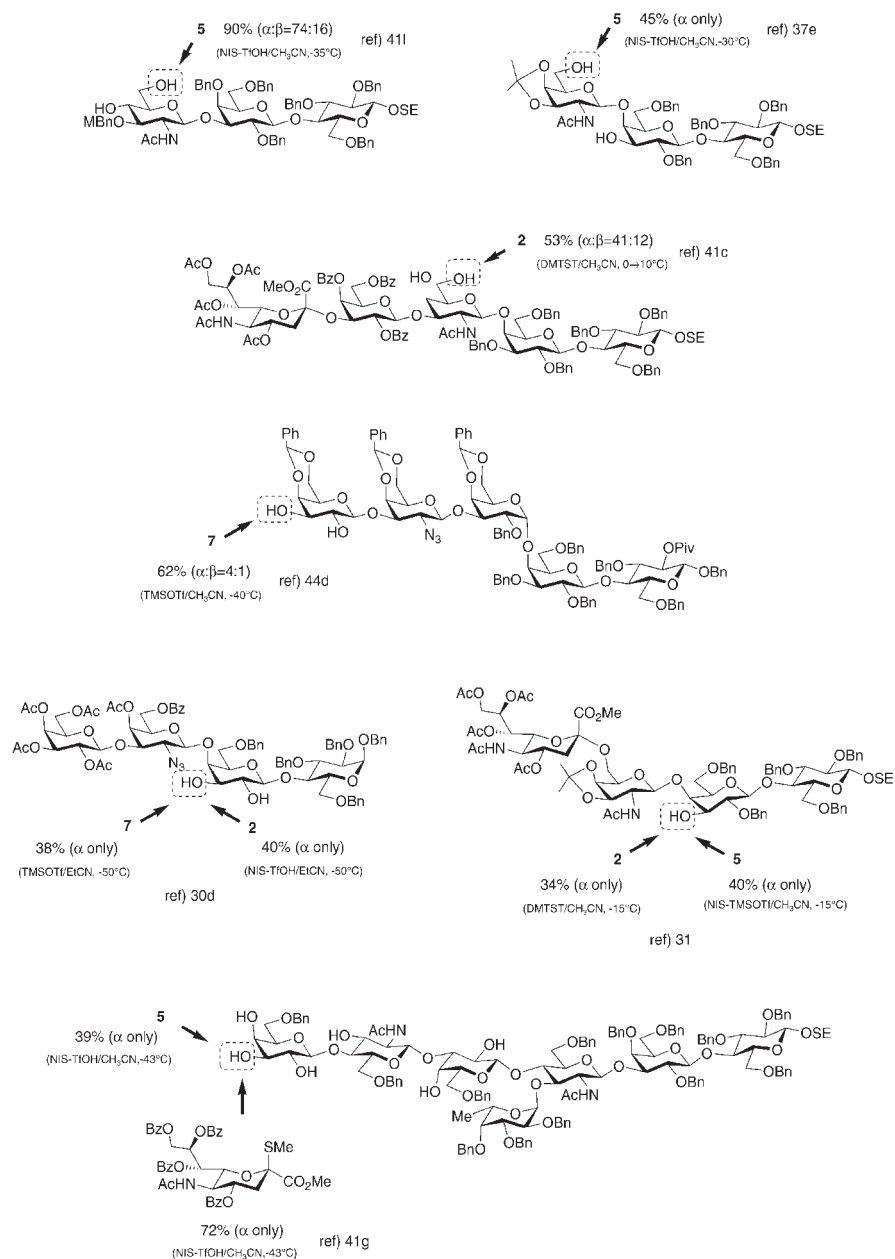


Figure 9.5

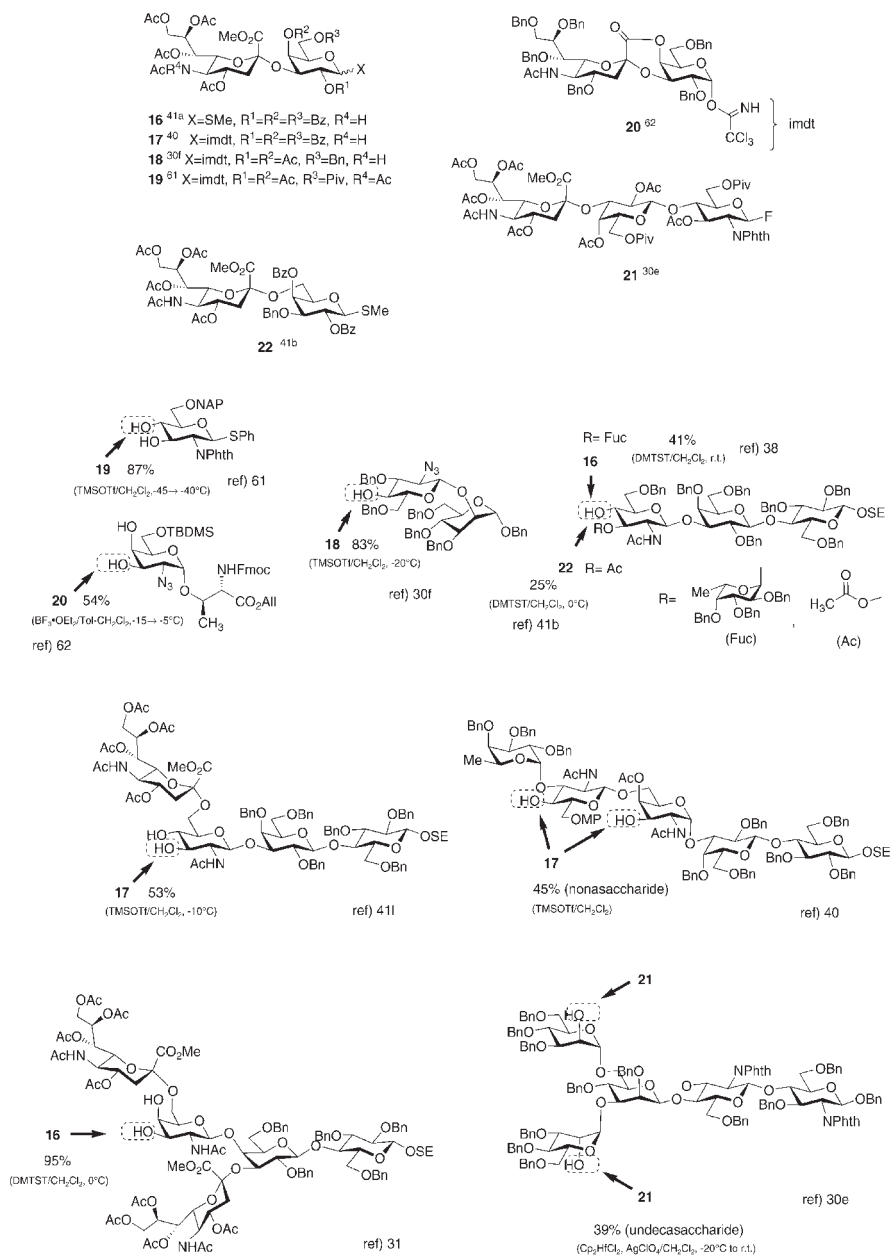
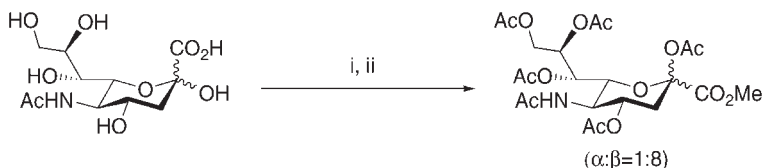


Figure 9.6

### 9.3.3 Experimental details

#### Method 1

Preparation of 2,4,7,8,9-penta-*O*-acetyl Neu5Ac derivative (modified protocol) [62].



i. Dowex-50(H<sup>+</sup>), MeOH, 40°C; ii. Ac<sub>2</sub>O, Pyr.

*Notes and discussion.* This protocol is applicable for the preparation of the title compound, which is a universal precursor to various types of sialic acid donors. Preparation of the acetyl derivatives can be performed on a large scale (~100 g of sialic acid) in the laboratory. Usually the resulting acetate is subjected to the next reaction after only an aqueous work-up. Although this procedure gives a mixture of α- and β-isomers in the ratio of 1 to 8, acetylation of the starting material with acetic anhydride-pyridine followed by esterification with diazomethane gives the β-acetate in 94%, which can be converted into the α-acetate via a two step-manipulation [9].

#### Materials.

<i>N</i> -Acetyl neuraminic acid (50 g, 0.16 mmol)	assume toxic
Cation exchange resin Dowex-50(H <sup>+</sup> ) (100 g)	irritant
Dry MeOH (500 ml, dried over MS 3 Å)	flammable, toxic
Calcium chloride	irritant
Acetic anhydride (153 ml, 1.6 mmol)	corrosive, lachrymator
Pyridine (179 ml, 2.4 mmol)	irritant
Toluene for co-evaporation (500 ml)	toxic, flammable
Ethyl acetate (1000 ml)	flammable, irritant
2 M HCl aqueous solution (200 ml)	irritant
Brine (200 ml)	assume toxic
Sat. NaHCO <sub>3</sub> aqueous solution (200 ml)	assume toxic
Sodium sulfate	irritant

*Equipment.*

Round bottomed flask (1000 ml) with stirrer bar  
Oil bath with temperature controller  
Pipettes  
Funnel  
Dropping funnel  
Separatory funnel  
Rotary evaporator

*Special precautions.* Dowex-50 ( $\text{H}^+$ ) should be pretreated with 10% HCl aq., then thoroughly washed with water and methanol thoroughly and air-dried.

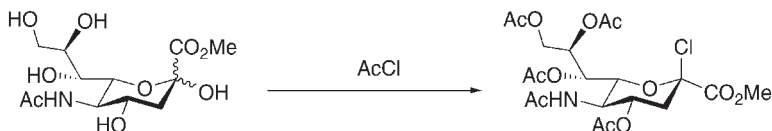
Add Dowex-50 ( $\text{H}^+$ ) (100 g) to a suspension of *N*-acetyl neuraminic acid (50 g, 0.16 mmol) in methanol and leave the mixture to stir at 40–45 °C overnight; the progress of the reaction is monitored by tlc analysis ( $\text{CHCl}_3/\text{MeOH} = 3:1$ ). In the course of the reaction the muddy mixture will turn clear. Filter off the resin and rinse with methanol several times in order to collect the product sticking to the resin. The combined filtrate and washings are evaporated *in vacuo* and exposed to high vacuum overnight.

Next, suspend the resulting residue in pyridine (179 ml, 2.4 mmol) in a 1000 ml flask, and add acetic anhydride (153 ml, 1.6 mmol) dropwise to the stirred suspension which is cooled by extrnal cooling with an ice-water bath. Stir the mixture overnight at ambient temperature—the reaction can be monitored by tlc analysis ( $\text{CHCl}_3/\text{MeOH} = 20:1$ ) (For the purpose of suppressing an explosive exothermic reaction, which could result for large scale preparations, it is also effective to add pyridine dropwise to the suspension of the resulting residue in acetic anhydride, in ice-water bath). Co-evaporate the reaction mixture with toluene to remove the remaining acetic anhydride, pyridine and acetic acid, dilute the syrupy mixture with ethyl acetate, and wash with 2 M HCl aq. (200 ml) and water. The aqueous layer should be washed once with ethyl acetate, and then wash the combined organic layer with sat  $\text{NaHCO}_3$  (200 ml) and brine (200 ml) and dry over  $\text{Na}_2\text{SO}_4$ . Filtration and evaporation of the mixture gives the crude material. Chromatographic purification can be conducted on silica gel to afford the pure title compound as a mixture of  $\alpha$ - and  $\beta$ -isomers ( $\alpha/\beta = 1/8$ ). The crude syrupy material is either dried prior to subjection to the next reaction, or can be lyophilized from dioxane for storage.

$\beta$ -acetate;  $[\alpha]_D = -32^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{C}_6\text{D}_6$ ) 5.62 (dd,  $J = 2.3, 3.8$  Hz, H-7, 1H), 5.46 (ddd,  $J = 2.3, 7.6$  Hz, H-8, 1H), 5.17 (ddd,  $J = 11.7, 10.5, 4.8$  Hz, H-4, 1H), 5.07 (dd,  $J = 12.4$  Hz, H-9a, 1H), 4.47 (ddd,  $J = 10.5$  Hz, H-5, 1H), 4.45 (dd, H-9b, 1H), 4.08 (dd, H-6, 1H), 3.45 (s, MeO, 3H), 2.57 (dd,  $J = 13.4$  Hz, H-3 $_{eq}$ , 1H), 1.95–1.61 (6s, 6Ac, 18H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ) 170.8, 170.2 170.1, 170.1, 168.1, 166.2, 97.3 (C-2), 72.7, 71.3, 68.2, and 67.7 (C-4,6–8), 62.0 (C-9), 53.0 (MeO), 49.0 (C-5), 35.8(C-3), 22.9, 20.7, 20.7, 20.6, 20.6 and 20.5 (6C $\text{H}_3\text{CO}$ ).

### Method 2

Preparation of 4,7,8,9-penta-*O*-acetyl-2-chloro- $\beta$ -Neu5Ac derivative [8].



**Notes and discussion.** The 2-chloride derivative can be prepared via several protocols [8, 9, 10, 11]. This protocol is the simplest and safest method, and allows facile large-scale preparation. The 2-chloro derivative is often used to prepare the 2,3-dehydro derivative [8b], which is pivotal for access to C-3 appended type donors. It is also central to strategies involving Koenigs–Knorr reactions with simple alcohols. Immediate use is recommended after preparation, despite the moderate shelf life of the title compound in the freezer.

### Materials.

Methyl <i>N</i> -acetyl neuramate (1.0 g, 1.8 mmol)	irritant
Acetyl chloride (10 ml, excess)	corrosive
Toluene for co-evaporation	toxic, flammable
Benzene for crystallization	cancer suspect agent, flammable
Diethyl ether for crystallization	toxic, flammable
Petroleum ether for crystallization	toxic, flammable

### Equipment.

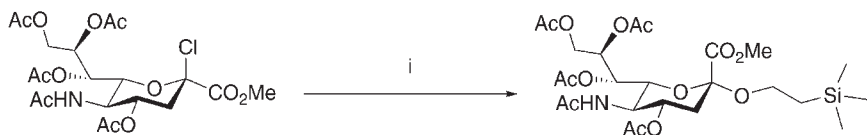
Round bottomed flask (50 ml) with stirrer bar  
Glass stopper  
Magnetic stirrer  
Pipette  
Rotary evaporator

**Special precautions.** All glassware must be dried prior to use. The reaction must be conducted in a fume hood. Operators should wear safety glasses and gloves.

Add acetyl chloride (10 ml, excess) to a flask containing methyl *N*-acetyl neuramate (1.0 g), and leave the solution stirring overnight at ambient temperature. Co-evaporation of the reaction mixture with toluene *in vacuo* affords 1.5 g (95%) of the title compound as a white powder. Crystallization from benzene–diethylether–petroleum ether gives 0.95 g (60%) of colorless fine needles; mp 116–118°,  $[\alpha]_D = -68^\circ$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 3.91 (s, MeO, 3H), 2.78 (dd, *J* = 5.0, 12.0 Hz, H-3<sub>eq</sub>, 1H), 2.10–1.92 (5s, 5Ac, 15H).

*Method 3*

Koenigs–Knorr reaction of methyl 4,7,8,9-tetra-*O*-acetyl-2-chloro- $\beta$ -Neu5Ac with 2-(trimethylsilyl)ethanol [12b].



i.  $(\text{CH}_3)_3\text{SiCH}_2\text{CH}_2\text{OH}$ ,  $\text{AgClO}_4$ ,  $\text{Ag}_2\text{CO}_3$ / MS 4Å,  $\text{CH}_2\text{Cl}_2$

*Notes and discussion.* Since the 2-(trimethylsilyl)ethyl glycoside of Neu5Ac is relatively stable to oxidative, reductive, acidic and basic treatment, it serves as a useful intermediate for the chemical derivation of sialic acid itself. In addition, the glycoside derivative is endowed with moderate lipophilicity by the 2-(trimethylsilyl)ethyl moiety which facilitates its handling.

*Materials.*

Methyl 4,7,8,9-tetra- <i>O</i> -acetyl-2-chloro- $\beta$ -Neu5Ac (10.2 g, 20 mmol)	assume toxic
2-(Trimethylsilyl)ethanol (5.7 ml, 40 mmol)	irritant
Silver carbonate (11 g, 53 mmol)	irritant, light-sensitive
Silver perchlorate (0.2 g, 0.72 mmol)	corrosive
4 Å molecular sieves (powder) (8 g, preactivated)	assume toxic
Dry dichloromethane (100 ml, dried over MS 4 Å)	toxic, irritant
Celite <sup>®</sup>	assume toxic

*Equipment.*

Round bottomed flask (500 ml, 200 ml each) with stirrer bar  
 Magnetic stirrer  
 Pipettes  
 Funnel  
 Suction filter  
 Rotary evaporator  
 Column chromatography equipment

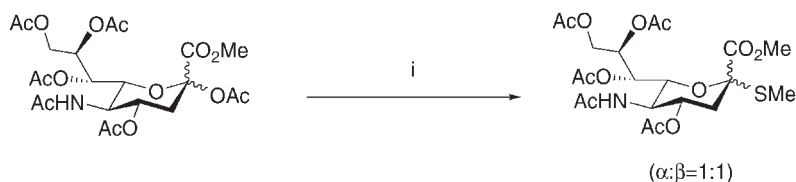
*Special precautions.* All glassware must be dried prior to use. The operator should wear safety glasses and gloves.

4 Å molecular sieves must be pre-activated by heating at 300 °C for 3 h. The reaction mixture should be shielded from light because of the light-sensitive properties of silver salts.

Stir two suspensions (A) and (B) for 5 h: (A) consists of methyl 4,7,8,9-tetra-*O*-acetyl-2-chloro- $\beta$ -Neu5Ac (10.2 g, 20 mmol) and 4 Å molecular sieves (4 g) in dichloromethane (50 ml) and (B) consists of 2-(trimethylsilyl)ethanol (5.7 ml, 40 mmol), silver carbonate (11 g, 53 mmol), silver perchlorate (0.2 g, 0.96 mmol) and 4 Å molecular sieves (4 g) in dichloromethane (50 ml). Then add suspension (A) into (B) with stirring and cooling via an ice-water bath. Then leave the mixture to stir at ambient temperature overnight. Filter off the precipitate through Celite<sup>®</sup> and wash with dichloromethane. The combined filtrate and washings are evaporated *in vacuo* to afford a syrup. Chromatographic purification of the residue on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 150:1) affords the title compound (10.5 g, 89%). Recrystallization from diethylether-hexane gives the product as needles; mp 84–86°, [ $\alpha$ ]<sub>D</sub> = –10° (*c* 0.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 5.39 (dd, H-8, 1H), 5.32 (dd, *J* = 1.7, 8.3 Hz, H-7, 1H), 4.83 (ddd, *J* = 12.7, 9.3 Hz, H-4, 1H), 4.30 (dd, *J* = 12.5 Hz, H-9a, 1H), 4.09 (dd, *J* = 2.7 Hz, H-9b, 1H), 4.05 (q, *J* = 9.3 Hz, H-5, 1H), 3.88 and 3.31 (2dd, TMSCH<sub>2</sub>CH<sub>2</sub>O, 2H), 3.79 (s, MeO, 3H), 2.57 (dd, *J* = 12.7, 4.4 Hz, H-3<sub>eq</sub>, 1H), 2.14–1.88 (5s, 5Ac, 15H) and 0.88 (m, TMSCH<sub>2</sub>CH<sub>2</sub>, 2H).

#### Method 4

Preparation of the methylthioglycoside of sialic acid [21b].



i. TMSSMe, TMSOTf/ MS 4Å, (CICH<sub>2</sub>)<sub>2</sub>, 50 °C

**Notes and discussion.** This reaction gives the 2-methylthioglycoside of sialic acid as a mixture of  $\alpha$ - and  $\beta$ -isomers in the ratio of 1:1 in a 96% yield. Originally the  $\alpha$ -methylthio derivative was synthesized from the corresponding 2-Cl derivative by treatment with potassium thioacetate, sodium methoxide, and methyl iodide [21a]. Since the anomeric ratio of methylthioglycoside donor does not affect the anomeric selectivity in coupling reactions, this short-step protocol is often utilized for the more practical preparation of the title compound.

#### Materials.

Methyl 2,4,7,8,9-penta- <i>O</i> -acetyl-Neu5Ac (40 g, 61 mmol)	assume toxic
(Methylthio)trimethylsilane (27 g, 225 mmol)	flammable, moisture-sensitive

Trimethylsilyl trifluoromethanesulfonate (13 g, 61 mmol)	flammable, corrosive
Dry 1,2-dichloroethane (600 ml, dried over MS 4 Å)	cancer suspect agent, flammable
4 Å molecular sieves (powder) (10 g)	assume toxic
1 M Na <sub>2</sub> CO <sub>3</sub> aqueous solution (500 ml)	
Sodium sulfate	irritant

*Equipment.*

Round bottomed flask (2000 ml) with stirrer bar  
 Magnetic stirrer  
 Pipettes  
 Funnel  
 Separatory funnel  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven prior to use. The reaction must be conducted in a fume hood. The operator should wear safety glasses and gloves.

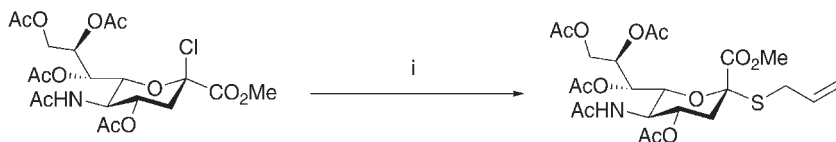
Add dry 1,2-dichloroethane (600 ml) to a flask containing methyl 2,4,7,8,9-penta-*O*-acetyl-Neu5Ac (40 g, 61 mmol). After addition of (methylthio)trimethylsilane (27 g, 225 mmol), trimethylsilyl trifluoromethanesulfonate (13 g, 61 mmol) and 4 Å molecular sieves (10 g) to the solution, leave the mixture under stirring for 6 h at 50 °C. The reaction progress can be monitored by tlc analysis (CHCl<sub>3</sub>/MeOH = 20:1). Then, add 1 M Na<sub>2</sub>CO<sub>3</sub> (500 ml) to the reaction mixture in an ice-water bath, separate the organic layer, wash it with water and dry (Na<sub>2</sub>SO<sub>4</sub>). Filtration and evaporation of the mixture *in vacuo* gives the crude residue, which is subjected to column chromatography on silica gel (toluene/MeOH = 70:1) by successive elution with (a) CH<sub>2</sub>Cl<sub>2</sub>, (b) 200:1 and (c) 50:1 CH<sub>2</sub>Cl<sub>2</sub>-MeOH to obtain the thioglycoside (37 g, 96%) as a mixture of α- and β-isomers (ratio 1:1).

α-Isomer; [26b] mp 80–82°, [ $\alpha$ ]<sub>D</sub> = –17° (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 5.38 (m, H-8, 1H), 5.33 (m, H-7, 1H), 4.88 (ddd, *J* = 10.2, 4.8 Hz, H-4, 1H), 4.33 (dd, *J* = 2.4 Hz, H-9a, 1H), 4.11 (dd, *J* = 11.7, 5.1 Hz, H-9b, 1H), 4.08 (q, *J* = 10.2 Hz, H-5, 1H), 3.84 (dd, *J* = 10.2, 1.8 Hz, H-6, 1H), 3.81 (s, MeO, 3H), 2.73 (dd, *J* = 12.6, 4.8 Hz, H-3*eq*, 1H), 2.17, 2.14 2.11, 2.04, 2.03 and 1.88 (6s, 6Ac, 18H).



*Method 5*

Stereoselective synthesis of allylthioglycoside under phase transfer catalytic conditions [20a].



i.  $\text{CH}_2=\text{CHCH}_2\text{SH}$ ,  $\text{Bu}_4\text{NHSO}_4$ /  $\text{EtOAc}$ , 1 M  $\text{Na}_2\text{CO}_2$  aq.

*Notes and discussion.* This reaction can be utilized for the preparation of  $\alpha$ -allyl, ethyl, phenyl and substituted aryl thioglycosides of sialic acid.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of the crude reaction mixtures have confirmed that complete anomeric inversion occurs under the PTC condition. Adoption of tetrabutylammoniumhydrogen sulfide as a PTC is critical for mild reaction conditions, otherwise, if triethylbenzylammonium chloride is employed, more harsh conditions are required. The use of ethyl acetate is advantageous over dichloromethane owing to the production of  $\text{PhSCH}_2\text{Cl}$  and  $(\text{PhS})_2\text{CH}_2$  resulting from nucleophilic displacement of one or two chlorides of dichloromethane with thiophenol in the model experiment.

*Materials.*

Methyl 4,7,8,9-tetra-*O*-acetyl-2-chloro- $\beta$ -Neu5Ac (118 mg, 0.232 mmol) assume toxic

Allyl mercaptan (75  $\mu\text{l}$ , 0.928 mmol) flammable, stench

Tetrabutylammonium hydrogen sulfate (78.8 mg, 0.232 mmol) assume toxic

Ethyl acetate (19.2 ml) flammable, irritant

1 M  $\text{Na}_2\text{CO}_3$  aqueous solution (1.2 ml) toxic, irritant

Sat.  $\text{NaHCO}_3$  aqueous solution (60 ml) assume toxic

Distilled water (20 ml)

Brine (10 ml) assume toxic

Sodium sulfate irritant

*Equipment.*

Round bottomed flask (30 ml) with stirrer bar

Magnetic stirrer

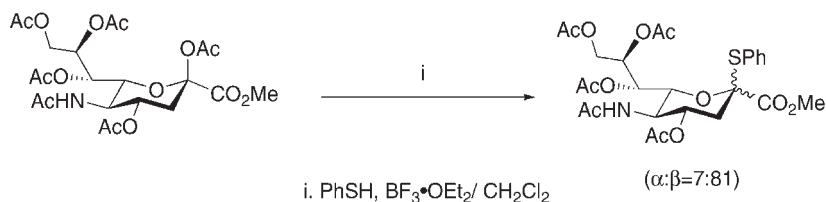
Pipettes  
Syringe  
Funnel  
Separatory funnel  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* The reaction must be conducted in a fume hood. The operator should wear safety glasses.

Add a solution of allyl mercaptan (75  $\mu$ l, 0.928 mmol) in aq. 1 M  $\text{Na}_2\text{CO}_3$  (1.2 ml) to a solution of methyl 4,7,8,9-tetra-*O*-acetyl-2-chloro- $\beta$ -Neu5Ac (118 mg, 0.232 mmol) and tetrabutylammonium hydrogen sulfate (78.8 mg, 0.232 mmol) in ethyl acetate (1.2 ml). Leave the mixture stirring vigorously for 1 h at room temperature. Dilute the reaction mixture with ethyl acetate (18 ml) and wash three times with sat.  $\text{NaHCO}_3$  aq. (20 ml each), twice with water (20 ml) and once with brine (10 ml), then dry the collected organic layer over  $\text{Na}_2\text{SO}_4$ . Filtration and evaporation of the mixture *in vacuo* gives the crude material, which is subjected to column chromatography on silica gel (EtOAc/hexane containing 0.5% isopropanol) to obtain the pure title compound as needles (106.4 mg, 84%); mp 108–111°,  $[\alpha]_{\text{D}} = +38^\circ$  (*c* 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 5.73 (m,  $J = 17.0, 10.0$  Hz,  $\text{CH}=\text{CH}_2$ , 1H), 5.37 (ddd,  $J = 8.2, 5.4, 2.7$  Hz, H-8, 1H), 5.29 (dd,  $J = 2.2$  Hz, H-7, 1H), 5.18 and 5.06 (m,  $=\text{CH}_2$ , 1H), 4.83 (ddd,  $J = 11.7, 10.4, 4.6$  Hz, H-4, 1H), 4.29 (dd,  $J = 12.5, 2.7$  Hz, H-9a, 1H), 4.09 (dd,  $J = 5.4$  Hz, H-9b, 1H), 4.02 (q,  $J = 10.7$  Hz, H-5, 1H), 3.76 (s, MeO, 3H), 2.69 (dd,  $J = 12.7, 4.6$  Hz, H-3eq, 1H).

#### Method 6

Preparation of the phenylthioglycoside of sialic acid [22a].



*Notes and discussion.* This reaction affords the 2-phenyl-, ethyl- and benzyl thioglycosides of sialic acid on exposure to the corresponding mercaptans with enhanced  $\beta$ -selectivity. In the literature, treatment of the  $\beta$ -penta-*O*-acetate derivative with thiophenol produces 81% of the  $\beta$ -phenylthioglycoside derivative together with 7% of the  $\alpha$ -isomer. In practice, the phenylthioglycoside is prepared from the mixture of  $\alpha$ - and  $\beta$ -penta-*O*-acetates (ratio ca. 1:8), which is subjected to the coupling reaction without fractionation of each isomer.

*Materials.*

Methyl 2,4,7,8,9-penta- <i>O</i> -acetyl-Neu5Ac (533 mg, 1.0 mmol)	assume toxic
Thiophenol (110 $\mu$ l, 1.1 mmol)	highly toxic, stench
Boron trifluoride diethyletherate (300 $\mu$ l, 2.5 mmol)	corrosive, moisture-sensitive
Dry dichloromethane (10 ml, dried over MS 4 Å)	toxic, irritant
Dichloromethane (150 ml)	toxic, irritant
Sat. NaHCO <sub>3</sub> aqueous solution (30 ml)	assume toxic
Brine (10 ml)	
Sodium sulfate	irritant

*Equipment.*

Round bottomed flask (30 ml) with stirrer bar  
 Magnetic stirrer  
 Pipettes  
 Funnel  
 Separatory funnel  
 Rotary evaporator  
 Column chromatography equipment

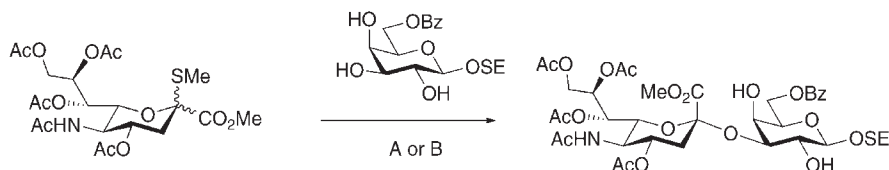
*Special precautions.* All glassware must be dried in an oven prior to use. The reaction must be conducted in a fume cupboard. The operator should wear safety glasses and gloves.

Add dry dichloromethane (10 ml) to a flask containing methyl 2,4,7,8,9-penta-*O*-acetyl-Neu5Ac (533 mg, 1.0 mmol) under an inert atmosphere. After addition of boron trifluoride diethyletherate (300  $\mu$ l, 2.5 mmol) to the solution (with external cooling for large-scale preparations), leave the mixture stirring overnight at room temperature. The reaction progress can be monitored by tlc analysis (CHCl<sub>3</sub>/MeOH = 30:1). Then, dilute the reaction mixture with dichloromethane (150 ml), wash with water (20 ml), sat. aq. NaHCO<sub>3</sub> (30 ml) and brine (10 ml) and dry the organic layer over Na<sub>2</sub>SO<sub>4</sub>. Filtration and evaporation of the mixture *in vacuo* gives the crude residue, which is subjected to column chromatography on silica gel (toluene/MeOH = 70:1) to obtain the  $\beta$ -thioglycoside (472 mg, 81%) and the  $\alpha$ -thioglycoside (40 mg, 7%).

$\beta$ -Isomer; mp 181–182°, [ $\alpha$ ]<sub>D</sub> = –132° (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.64, 7.10 and 6.99 (3m, Ph, 5H), 5.72 (dd, *J* = 2.5, 2.1 Hz, H-7, 1H), 5.44 (ddd, *J* = 8.6, 2.1 Hz, H-8, 1H), 5.35 (ddd, *J* = 11.7, 10.2, 4.8 Hz, H-4, 1H), 5.00 (dd, *J* = 12.4 Hz, H-9a, 1H), 4.61 (m, H-6, 1H), 4.38 (dd, H-9b, 1H), 3.26 (s, MeO, 3H), 2.80 (dd, *J* = 14.0 Hz, H-3*eq*, 1H), 2.01 (dd, H-3*ax*, 1H), 1.92, 1.88 1.67, 1.63 and 1.60 (5s, 5Ac, 15H).

*Method 7*

Coupling reaction of the 2-methylthioglycoside of sialic acid with 2-(trimethylsilyl)ethyl 6-*O*-benzoyl- $\beta$ -D-galactopyranoside in acetonitrile [26, 27].



A. DMTST/ MS 3 Å, CH<sub>3</sub>CN, -40  $\rightarrow$  -15°C  
 B. NIS-TfOH/ MS 3 Å, CH<sub>3</sub>CN, -40°C

*Notes and discussion.* This coupling reaction can be promoted by either dimethyl(methylthio)sulfonium triflate (DMTST) [25, 26] or *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH) [27] in acetonitrile to predominantly afford the  $\alpha$ -sialoside. The excellent anomeric selectivity is a consequence of solvent participation under thermodynamically controlled conditions, and the high yield is due to minimal protection of the galactose acceptor. Otherwise, if the corresponding 2,6-di-*O*-benzoyl derivative is employed, poor results can be obtained. For large quantity preparations the corresponding 2-phenylthioglycoside derivatives can be used as donor and NIS–TfOH as the promoter. This combination offers the advantage of lower cost and a better safety profile compared with the reaction of the 2-methylthioglycoside and DMTST.

*Materials.*

For DMTST-promoted glycosylation:

2-Methylthioglycoside derivative of Neu5Ac (430 mg, 0.824 mmol) assume toxic

$\beta$ -D-Galactopyranoside derivative (160 mg, 0.416 mmol) assume toxic

DMTST mixed with MS 3 Å [25c] (1.7 g, 3.29 mmol; 50% w/w) highly toxic

3 Å molecular sieves (powder) (400 mg) assume toxic

Dry acetonitrile (2.5 ml, dried over MS 3 Å) flammable, toxic

Celite<sup>®</sup> assume toxic

Dichloromethane (50 ml) toxic, irritant

Sat. Na<sub>2</sub>CO<sub>3</sub> aqueous solution (15 ml) toxic, irritant

Sodium sulfate irritant

For NIS–TfOH-promoted glycosylation:

2-Methylthioglycoside derivative of Neu5Ac (1.6 g, 3.2 mmol)	assume toxic
$\beta$ -D-Galactopyranoside derivative (2.4 g, 1.9 mmol)	assume toxic
NIS (1.4 g, 6.4 mmol)	moisture-sensitive, irritant
TfOH (56 $\mu$ l, 0.64 mmol)	corrosive, hygroscopic
3 Å molecular sieves (powder) (2.3 g)	assume toxic
Dry acetonitrile (15 ml, dried over MS 3 Å)	flammable, toxic
Celite <sup>®</sup>	assume toxic
Dichloromethane (150 ml)	toxic, irritant
Sat. Na <sub>2</sub> CO <sub>3</sub> aq. (30 ml)	toxic, irritant
Sat. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> aq. (30 ml)	irritant
Sodium sulfate	irritant

#### *Equipment.*

Round bottomed flask (30 ml) with stirrer bar  
Magnetic stirrer  
Pipettes  
Syringe  
Suction filter  
Separatory funnel  
Rotary evaporator  
Column chromatography equipment

*Special precautions.* All glassware must be dried in an oven prior to use. The operator should wear safety glasses and gloves.

3 Å molecular sieves must be pre-activated by heating at 300 °C for 3 h.

*DMTST-promoted glycosylation:* Add dry acetonitrile (15 ml) to a flask containing methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-nonulopyranosid)onate (430 mg, 0.824 mmol), 2-(trimethylsilyl)ethyl 6-*O*-benzoyl- $\beta$ -D-galactopyranoside (160 mg, 0.416 mmol) and 3 Å molecular sieves (400 mg) under an inert atmosphere. After the suspension is stirred for 5 h and cooled to –40 °C, add the mixture of DMTST and 3 Å molecular sieves (1.7 g; 50% of DMTST by weight) to the suspension and then leave the mixture to stir at –15 °C for 15 h; The reaction progress can be monitored by tlc analysis (CHCl<sub>3</sub>/MeOH = 15:1). Then, filter off the precipitate through Celite<sup>®</sup> and wash with dichloromethane. Aqueous work-up of the combined filtrate and washings with sat. aq. Na<sub>2</sub>CO<sub>3</sub> (30 ml) and drying of the resulting organic layer over

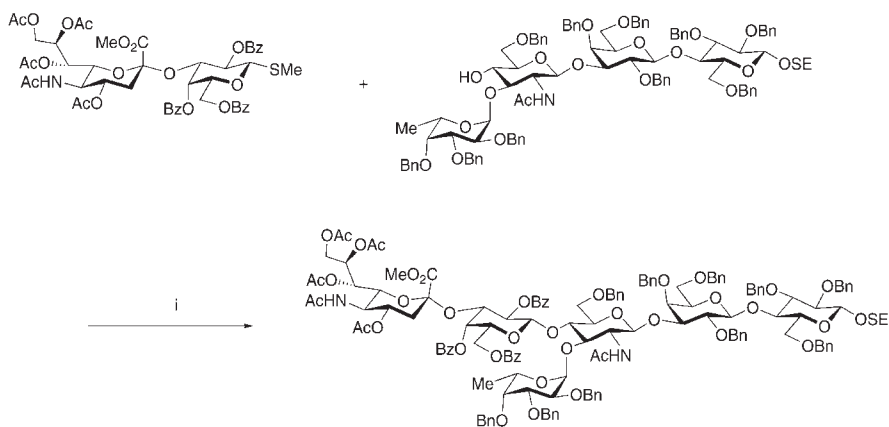
$\text{Na}_2\text{SO}_4$  is followed by filtration and evaporation *in vacuo* to give the crude mixture. Column chromatography of the residue on silica gel (toluene/MeOH = 70:1) then affords the title compound (154 mg, 43%) as an oil.

**NIS–TfOH-promoted glycosylation:** Add dry acetonitrile (15 ml) to a flask containing methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-nonulopyranosid)onate (1.6 g, 3.2 mmol), 2-(trimethylsilyl)ethyl 6-*O*-benzoyl- $\beta$ -D-galactopyranoside (2.4 g, 1.9 mmol) and 3 Å molecular sieves (2.3 g) under an inert atmosphere. After the suspension is stirred for 5 h and cooled to  $-40^\circ\text{C}$ , add NIS (1.4 g, 6.4 mmol) and TfOH (53  $\mu\text{l}$ , 0.64 mmol) to the suspension, and continue to stir at  $-40^\circ\text{C}$  for 2 h. The reaction progress can be monitored by tlc ( $\text{CHCl}_3/\text{MeOH} = 15:1$ ). Filter off the precipitate through Celite® and wash with dichloromethane, wash the combined filtrate and washings with sat. aq.  $\text{Na}_2\text{CO}_3$  (30 ml) and sat. aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (10 ml), and dry the organic layer over  $\text{Na}_2\text{SO}_4$ . Filtration and evaporation of the mixture *in vacuo* gives the crude residue, which is subjected to column chromatography on silica gel (toluene/MeOH = 70:1) to afford the title compound (472 mg, 61%) as an oil.

$[\alpha]_{\text{D}} = -6^\circ$  (*c* 2.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3\text{--CD}_3\text{OD} = 1:1$ ) 8.13–7.40 (m, Ph, 5H), 5.45 (ddd,  $J = 4.8, 2.2$  Hz, H-8<sup>Neu</sup>, 1H), 5.33 (dd,  $J = 8.3, 3.6$  Hz, H-7<sup>Neu</sup>, 1H), 4.95 (ddd,  $J = 11.4, 9.7$  Hz, H-4<sup>Neu</sup>, 1H), 4.62 (dd,  $J = 5.3$  Hz, H-6a<sup>Gal</sup>, 1H), 4.54 (dd,  $J = 11.4, 6.1$  Hz, H-6b<sup>Gal</sup>, 1H), 4.46 (d,  $J = 4.9$  Hz, H-1<sup>Gal</sup>, 1H), 4.27 (dd,  $J = 12.5, 2.2$  Hz, H-9a<sup>Neu</sup>, 1H), 3.78 (s, MeO, 3H), 2.72 (dd,  $J = 12.8, 4.8$  Hz, H-3eq<sup>Neu</sup>, 1H), 2.12–1.90 (5s, 5Ac, 15H).

### Method 8

Coupling reaction of an  $\alpha$ -(2  $\rightarrow$  3)sialyl-galactose donor and a tetrasaccharide acceptor to afford the sialyl Lewis X hexasaccharide [38a].



i. DMTST/MS 4Å,  $\text{CH}_2\text{Cl}_2$ , r. t.

*Notes and discussion.* Coupling reactions that exploit the methylthioglycoside of  $\alpha$ -(2  $\rightarrow$  3)sialyl-galactose derivative have produced a number of sialo-oligosaccharides to date. For glycosylations with less reactive acceptors, activation by the action of dimethyl(methylthio)sulfonium trifluoromethanesulfonate is considered to be more feasible. Recently the corresponding trichloroacetimidate donor has also been utilized.

*Materials.*

Methylthioglycoside of $\alpha$ -(2 $\rightarrow$ 3)sialyl-galactose derivative (417 mg, 0.42 mmol)	assume toxic
Tetrasaccharide derivative (473 mg, 0.28 mmol)	assume toxic
DMTST mixed with MS 4 Å (650 mg, 1.26 mmol; 50% w/w)	highly toxic
4Å molecular sieves (powder) (1.22 g)	assume toxic
Dry dichloromethane (8 ml, dried over MS 4 Å)	toxic, irritant
Triethylamine (0.5 ml)	flammable, corrosive
Celite <sup>®</sup>	assume toxic
Dichloromethane (50 ml)	toxic, irritant
Distilled water (15 ml)	
Sodium sulfate	irritant

*Equipment.*

Round bottomed flask (30 ml) with stirrer bar  
 Magnetic stirrer  
 Pipettes  
 Suction filter  
 Separatory funnel  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions.* All glassware must be dried prior to use. The operator should wear safety glass and gloves.

4 Å molecular sieves must be pre-activated by heating at 300 °C for 3 h.

Add dry dichloromethane (8 ml) to a flask containing the donor (417 mg, 0.42 mmol), acceptor (473 mg, 0.28 mmol) and 4 Å molecular sieves (1 g). After the suspension has stirred for 4 h at room temperature, add the mixture of DMTST (325 mg, 1.26 mmol) and 4 Å molecular sieves (220 mg) to the suspension. Then leave the mixture to stir at room temperature for 20 h. The progress of the reaction

is monitored by tlc analysis ( $\text{CHCl}_3/\text{MeOH} = 10/1$ ). Quench DMTST by the addition of methanol (1 ml) and triethylamine (5 ml), and filter off the precipitate through Celite® and wash with dichloromethane. Aqueous work-up of the combined filtrates and washings with water and drying of the resulting organic layer over  $\text{Na}_2\text{SO}_4$  is followed by filtration and evaporation *in vacuo* to give the crude mixture. Column chromatography of the residue on silica gel ( $\text{EtOAc}/\text{hexane} = 4:1$ ) affords the title compound (300 mg, 41%) as an oil;  $[\alpha]_{\text{D}} = -14^\circ$  ( $c$  0.7,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 8.19–7.05 (m, 13Ph, 65H), 5.67 (m, H-8<sup>Neu</sup>, 1H), 5.43 (dd,  $J = 9.9, 8.1$  Hz, H-2<sup>Gal</sup>, 1H), 5.32 (broad d  $J = 3.1$  Hz, H-4<sup>Gal</sup>, 1H), 5.21 (dd,  $J = 12.1, 2.7$  Hz, H-7<sup>Neu</sup>, 1H), 3.77 (s, MeO, 3H), 2.42 (dd,  $J = 12.4, 4.6$  Hz, H-3<sup>eq</sup><sub>Neu</sub>, 1H), 2.42–1.45 (6s, 6Ac, 18H), 1.06 (d,  $J = 6.6$  Hz, H-6<sup>Fuc</sup>, 3H), 1.00 (m,  $\text{TMSCH}_2\text{CH}_2$ , 2H).

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## The Synthesis of Glycosyl Amino Acids

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### 10.1 INTRODUCTION

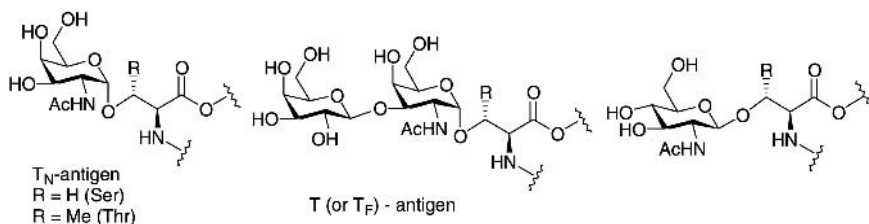
Most naturally occurring proteins display *O*-linked glycopeptides and *N*-linked glycopeptides and have immense biological significance [1]. Several reviews and texts cover the most recent progress in glycopeptide synthesis [2]. Since the isolation of glycopeptides from natural sources is difficult, they are often prepared by chemical synthesis [3] employing a combination of both carbohydrate and peptide chemistry. This chapter details the synthesis of glycosyl amino acids of use in glycopeptide synthesis. However, the enzymic [4], and solid phase synthesis [5] of glycopeptides are discussed elsewhere.

### 10.2 *O*-GLYCOSYLAMINO ACIDS AND *O*-GLYCOPEPTIDES

*O*-Linked glycopeptides offer a more diverse range of compounds than the *N*-linked glycopeptides [6]. Thus although *N*-glycopeptides mainly incorporate L-asparagine (Asn) at the anomeric position, the *O*-glycopeptides involve anomeric linkages to L-threonine (Thr) and L-serine (Ser). A detailed review describes the complexity of the preparation of a wide range of *O*-linked glycosyl amino acids [7].

The most common *O*-glycopeptide core fragment contains a D-GalNAc unit that is  $\alpha$ -linked to serine or threonine (Figure 10.1) [8]. Such glycopeptides are located in mucins, found mainly in various sera and cell membrane glycoproteins. Different core regions have been identified that contain mainly GlcNAc, Gal or GalNAc carbohydrate residues. For example,  $\beta$ -D-GlcNAc attached to serine is

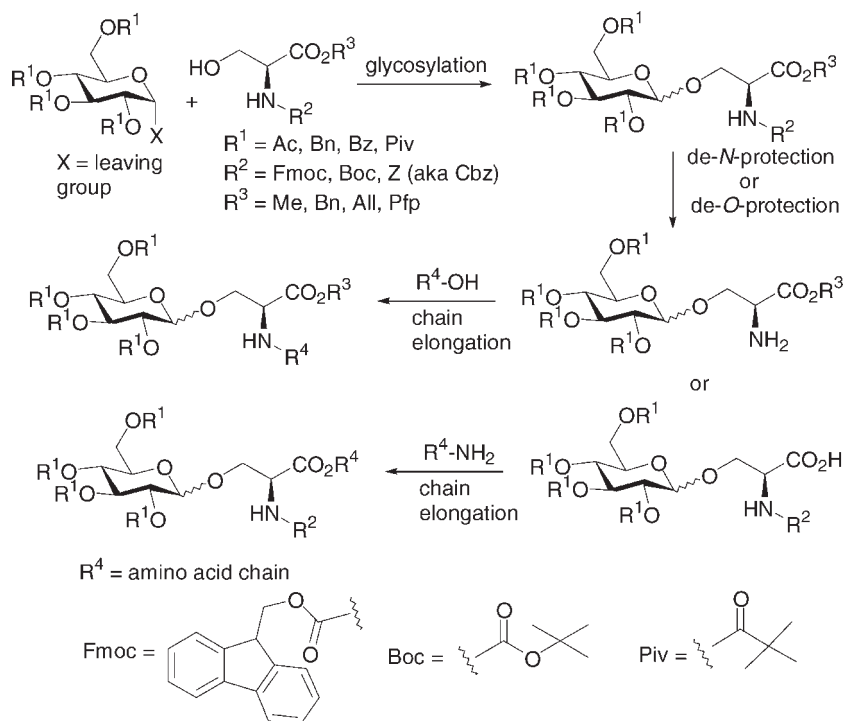




**Figure 10.1** GalNAc $\alpha$ -O-Ser/Thr, Gal $\beta$ (1  $\rightarrow$  3)-GalNAc $\alpha$ -O-Ser/Thr and GlcNAc $\beta$ -O-Ser.

found in nuclear pore proteins, cytoskeletal proteins and transcription factors. The disaccharide Gal $\beta$ -(1  $\rightarrow$  3)-GalNAc  $\alpha$ -O-linked to serine or threonine in mucins, is classified as a core 1 glycan and is a carcinoma associated Thomsen–Freidenreich ( $T_F$ )-antigen. Cancer mucins express this disaccharide in abnormally high quantities thus providing a target for cancer therapy. Some examples of *O*-linked  $\alpha$ -D-mannopyranosyl residues bound to amino acids have been reported, and are mainly found in glycoproteins from yeasts and moulds [9].

Synthesis of *O*-glycoamino acids, hence glycopeptides, requires glycosylation between a suitably protected amino acid and an activated carbohydrate donor (Scheme 10.1). *O*-Glycopeptides are best prepared by chain elongation at either



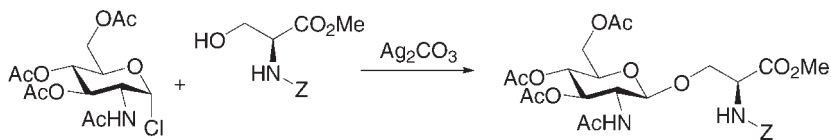
**Scheme 10.1** General elaboration of glycoamino acids  $\rightarrow$  glycopeptides.

the C- or N-terminus of the amino acid after removal of the amino/carboxyl protecting groups [10]. An alternative to this would be to synthesize the core peptide containing the hydroxy amino acid and subsequently perform a glycosylation reaction. However, this is known to be a more complex procedure, giving lower yields [11]. Before the glycosylation reaction can be performed, it is important to choose appropriate orthogonal protecting group combinations for the amino and carboxyl groups contained within the L-serine and L-threonine amino acids and the carbohydrate moiety. For example, incorporation of 9-fluorenylmethoxycarbonyl (Fmoc) and benzyl (Bn) protected amino acids allow versatile synthesis of homologated glycopeptides via deprotection of the Fmoc group by base, e.g. 50% morpholine in *N,N*-dimethylformamide (DMF) [12], and condensation with a suitably protected peptide (e.g.  $R^4-OH = Fmoc-Asn-Leu-OH$ ). However, with strong bases, *O*-glycopeptides can undergo  $\beta$ -elimination but fortunately they are stable to mild bases, such as morpholine, piperidine and 1,5-diazabicyclo[4.3.0]non-5-ene (DBU), thereby allowing the Fmoc group to be efficiently removed. Hydrazine in methanol (1:7) has been used to remove the benzoyl (Bz) groups [13]. The benzyl groups are removed by hydrogenolysis using  $H_2$ , Pd/C. *O*-Glycopeptides are also reasonably stable to acids for short periods, for example, trifluoroacetic acid (TFA) can be used to remove *t*-butyl groups and promote the cleavage of *O*-glycopeptides from solid phase resins. However, the glycosidic bond is somewhat acid sensitive resulting in hydrolysis and anomerization of the target. Interestingly, incorporation of acetyl protecting groups (which are electron withdrawing) within the carbohydrate helps to stabilize the *O*-glycosidic linkages during treatment with, for example, TFA. This is important for incorporation of 6-deoxy-L-fucose within synthetic strategies, as this is susceptible to acid catalysed hydrolysis. Acetate protecting groups are therefore frequently used and are easily removed with sodium methoxide in methanol with no epimerization of the amino acid or  $\beta$ -elimination being detected [14]. Occasionally attempts to glycosylate *tert*-butoxycarbonyl (Boc) protected serine or threonine compounds have failed due to the presence of trace amounts of free amino acid which results from partial hydrolysis of the Boc substituent. The presence of base, such as pyridine or collidine, suppresses the glycosylation reaction. No such problems have been reported with the Fmoc group. It should also be noted that the use of metals or their salts, for example zinc in DMF, for deprotection reactions should be avoided, as lower transformation yields are incurred due to the adsorption of the glycopeptides on to the surface of the metal or the salts [15].

### 10.2.1 Chemical synthesis of *O*-glycoamino acids and *O*-glycopeptides

The Koenigs–Knorr method remains one of the simplest glycosylation procedures. One relevant example includes treating *N*-acetylglucosamine chloride with *N*-(benzyloxycarbonyl)-L-serine methyl ester in the presence of silver carbonate and this affords the  $\beta$ -linked product (Scheme 10.2) [16]. However,

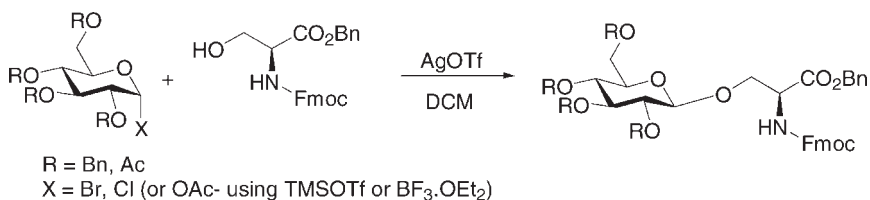




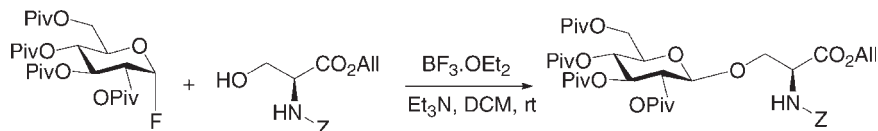
**Scheme 10.2** Koenigs–Knorr glycosylation.

classical Koenigs–Knorr reaction conditions often afford the product in poor yield due to the low nucleophilicity of the hydroxyl group of serine (or threonine). This can be improved by using a different amine protecting group or  $\alpha$ -imino ester (Schiff base) derivative of the amino acid [17]. Similarly, using a more reactive promoting reagent such as silver triflate, and utilizing Fmoc and benzyl protecting groups allows reaction of 2,3,4-tri-*O*-benzyl- $\alpha$ -D-glycosyl bromide with *N*-Fmoc-L-serine benzyl ester to give the 3-*O*-( $\beta$ -glucosyl)-L-serine derivatives in good yields (Scheme 10.3) [12, 18]. Activation of the 1-*O*-acetyl group (Scheme 10.3 X = OAc) can be promoted by addition of trimethylsilyl triflate (TMSOTf) [19] or BF<sub>3</sub>·OEt<sub>2</sub> [20]. By using promoters such as the Lewis acids BF<sub>3</sub>·OEt<sub>2</sub> or SnCl<sub>4</sub>, it is even possible to perform the glycosylation of Fmoc protected amino acids containing the unprotected carboxylic acid, although this is less common [21]. Higher yields can also be achieved by using a non-participating group such as an azide at C-2. Therefore, using silver carbonate and silver perchlorate with 2-azido-2-deoxy-D-galactose and Fmoc protected serine or threonine, allows preparation of the  $\alpha$ -glycoside in good yield [22]. Protection of the amine moiety of the amino acid as the Fmoc carbamate, and the carboxylic acid as the pentafluorophenyl (Pfp) ester, appear to be most effective [23]. Pfp esters have multiple advantages as they protect the carboxylic acid during glycosylation and activate the carboxyl group for subsequent amide bond formation.

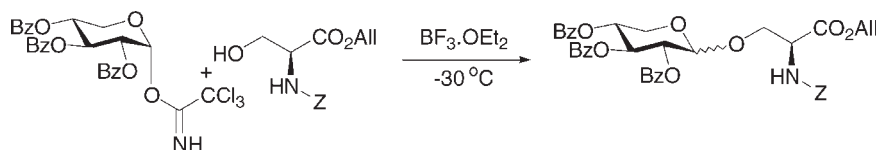
An alternative to the Koenigs–Knorr glycosylation method is to use the hydrolytically stable glycosyl fluoride donor which reacts, for example, with *N*-(benzyloxycarbonyl)-L-serine allyl ester [24] in the presence of excess boron trifluoride etherate to give the  $\beta$ -glycoamino-ester (1:5,  $\alpha/\beta$ ) (Scheme 10.4) [25]. Excess BF<sub>3</sub>·OEt<sub>2</sub> was used due to complexation with the pivaloyl esters. This could be avoided by using different hydroxyl protecting groups such as benzyl ethers in which case only a catalytic quantity of BF<sub>3</sub>·OEt<sub>2</sub> (0.2 equiv.) was necessary. Treatment of the trichloroacetimidate derivative [26] with boron



**Scheme 10.3**



Scheme 10.4

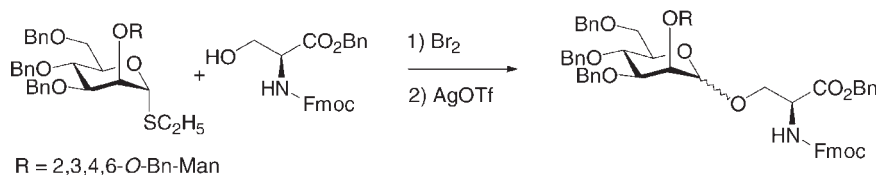


Scheme 10.5

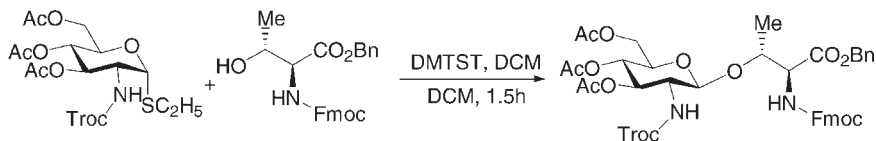
trifluoride and protected amino-ester affords the L-serine derivative as a mixture of  $\beta$ - and  $\alpha$ -glycosides with the  $\beta$ -form predominating (Scheme 10.5) [24]. TMSOTf has also been used to promote this reaction [27]. Z/Bn protected serine has similarly been used with trichloroacetimidate donors and boron trifluoride to give the  $\beta$ -glycoside in good yield [28]. The allyl protecting group can be selectively removed with catalytic tetrakis(triphenylphosphine) palladium(0) in THF with morpholine at room temperature in almost quantitative yield. Notably Fmoc is also cleaved by morpholine, hence the use of the *N*-benzyloxycarbonyl protecting group.

Interestingly, with a participating group such as the *N*-acetyl moiety, steric control is possible. For example, the 4,6-*O*-benzylidene-*N*-acetylgalactosamine trichloroacetimidate donor has been shown to react with serine or threonine using  $\text{BF}_3 \cdot \text{OEt}_2$  as promoter, to selectively form  $\alpha$ -glycosides [29]. Conversely the C-1 acetoxyl compound (2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranose) reacts under similar conditions to exclusively afford the  $\beta$ -glycoside [30].

Glycosylation can be efficiently achieved using the reactive thioglycosides as donors and these are readily prepared from the acetate glycoside via boron trifluoride catalysed reaction with ethane thiol in DCM 0 °C. Treatment of the ethyl 1-thio disaccharide with bromine (forming the  $\alpha$ -bromo donor) followed by activation with silver triflate in the presence of molecular sieves and reaction with Fmoc-L-Ser or Thr benzyl ester gives the  $\alpha$ -glycopeptide in 67% yield and the  $\beta$ -anomer in 12% (Scheme 10.6) [31]. Similarly, thiophilic activation has been achieved with dimethyl(methylthio)sulfoniumtrifluoromethane sulfonate (DMTST) [32] which

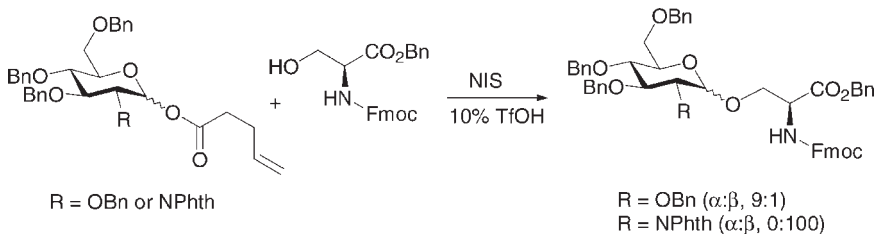


Scheme 10.6

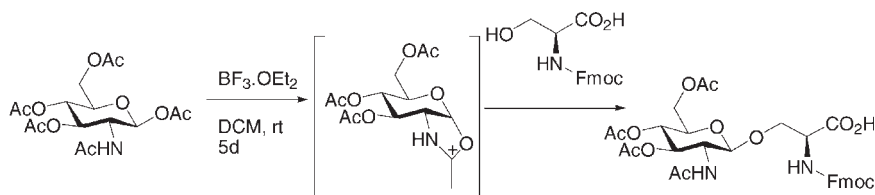


**Scheme 10.7** Activation of thioglycosyl donor.

gives, for example, the  $\beta$ -glycoside with Fmoc-Thr-OBn (Scheme 10.7) [33, 34] or methyl triflate (MeOTf) [35]. Sialic acid containing glycopeptides have been similarly prepared [36]. One disadvantage of this method is that it may be necessary to extensively purify the product depending on the next synthetic step. For example, purification by HPLC has proved necessary to remove potential enzyme inhibitors [37]. Using milder reaction conditions, *n*-pentenyl glycosides can be activated by electrophiles ( $\text{Hal}^+$ ) such as *N*-iodosuccinimide (NIS) with triflic acid, or iodonium di-*sym*-collidine perchlorate (IDCP), or 1,3-dithian-2-ylum tetrafluoroborate in the presence of molecular sieves. Subsequent reaction with Fmoc-L-serine-benzyl ester affords the glycoamino acid in 69% with an  $\alpha/\beta$  ratio of 9:1 (Scheme 10.8) [38]. Stereoselectivity can be achieved by incorporation of a participating group at C-2, e.g. phthalimide (Phth). This method is most useful for the glycosylation of preformed peptides. Notably, this example contains a protected-*N*-carbohydrate. By using a suitable nitrogen protecting group or precursor (e.g. an azide) the glycosidic bond can be stereoselectively formed. This methodology has been discussed in a recent review [39]. If the glycosyl donor carries a participating group on the nitrogen (e.g. Ac, phthaloyl, 2,2,2-trichloroethoxycarbonyl (Troc-sometimes called Teoc)) [40], or dithiasuccinoyl (Dts) [41] or uses a 1,2-oxazoline donor, the  $\beta$ -glycoside is favored. To form the  $\alpha$ -glycoside, a non-participating group is employed, such as an azide. Under standard procedures, this can be reduced by hydrogenolysis over Pd/C and acetylated (pyridine and  $\text{Ac}_2\text{O}$ ) to give the acetamido group, or reduced with thioacetic acid. The *N*-acetyl group can be used to effect stereoselective glycoside bond formation via the reactive methyl oxazolinium ion intermediate (Scheme 10.9) [33, 41, 42]. After activation of the donor by the addition of excess Lewis acid, and stereoselective nucleophilic attack by the acceptor from the  $\beta$ -face, the pure *trans*- $\beta$ -glycoside is formed. Care should always be taken in such reactions since the *N*-acetyl moiety can form relatively stable oxazolines and then require elevated temperature to



**Scheme 10.8** NIS/TfOH is the reagent of choice for activating 4-pentenyl glycosides.



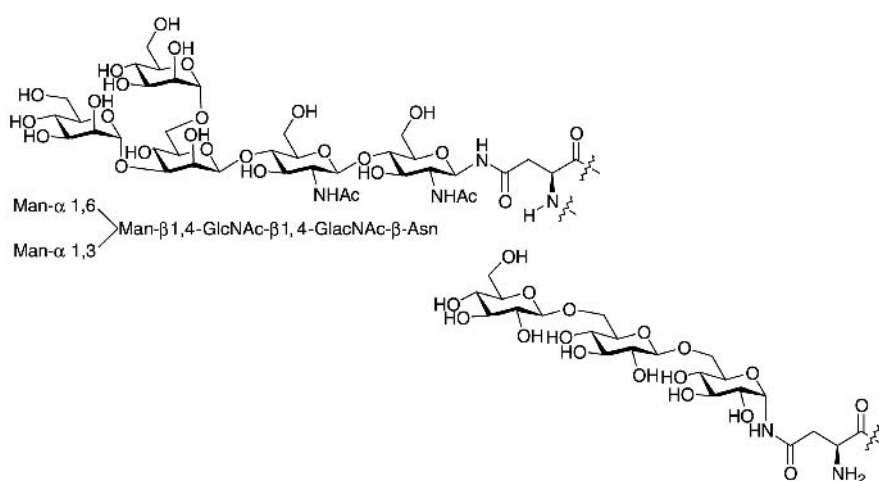
**Scheme 10.9** Glycosylation of Fmoc-Ser-OH to give  $\beta$ -glycoside.

react. In such cases it is also possible that the carboxyl group is glycosylated instead of the anomeric hydroxyl group [33].

### 10.3 *N*-GLYCOSYLAMINO ACIDS AND *N*-GLYCOPEPTIDES

*N*-Glycopeptides that are naturally occurring are  $\beta$ -*N*-linked to an *N*-acetyl glucosamine segment of a chitobiose pentasaccharide core. These are part of a high mannose antennary structure. The most common *N*-glycopeptide core fragment is derived from the  $\beta$ -linked asparagine-glucosamine (Asn-GlcNAc) (Figure 10.2) [43].

Examples that do not display the typical core  $\beta$ -D-glucosamine unit attached to an Asn side chain have been characterized [44], for example nephritogenoside that contains a glucose moiety  $\alpha$ -linked to the amide was isolated from the glomerular basement membrane of rats (Figure 10.2) [45].



**Figure 10.2**

### 10.3.1 Chemical synthesis of *N*-glycoamino acids and *N*-glycopeptides

As with *O*-glycopeptides there are two main approaches for the synthesis of complex *N*-glycopeptides, the stepwise approach and the convergent approach. In the former pathway, the most common approach employs a glycosyl asparagine analogue as an intermediate. Elaboration of the peptide sequence is usually carried out on a solid support. The versatility of the reaction allows a variety of carbohydrates and peptides to be included. A disadvantage of the stepwise pathway is the prerequisite for protection of the *N*- $\alpha$ -amino and *C*- $\alpha$ -carboxyl functions of the Asn moiety, in order to prevent formation of aspartimide. However, the carboxylic acid side chain of Asn should remain unprotected to effect *N*-glycoside formation. In the convergent approach formation of the amide linkage between the complete carbohydrate chain and the desired peptide sequence is carried out near the end of the synthesis. Both the carbohydrate and peptide segments are synthesized independently.

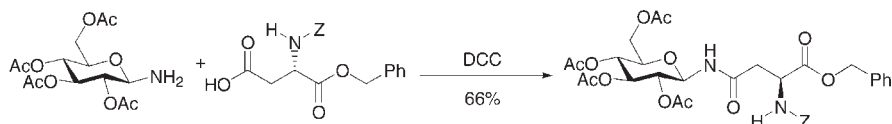
### 10.3.2 Formation of the *N*-glycosidic bond

The chemical synthesis of *N*-glycoamino acids and *N*-glycopeptides requires a completely different approach to the synthesis of *O*-glycoamino acids as an amide bond must be formed at the anomeric position. Therefore the chemistry more closely resembles amide bond formation than glycosylation chemistry. A range of glycosyl precursors have been employed to prepare *N*-glycosides, including anomeric glycosylamines, anomeric isothiocyanates, anomeric *n*-pentenyl glycosides and anomeric glycosyl azides. The formation of the *N*-glycosidic bond is often achieved by the reaction of a glycosylamine and an activated Asp derivative that has been suitably protected to avoid side reactions. The remainder of this section discusses the formation of the *N*-glycosidic bond using these glycosyl precursors.

#### *Anomeric glycosylamines*

The reaction of unprotected glycosylamines with suitably protected Asn residues has been reported [46] but the peptide bond formation is lower yielding than when protected glycosylamines are employed. A range of precursors has been utilized to access the glycosylamines such as oxazolines, halides and glycals. Glycosyl bromides have served as precursors, by reaction with ammonia [47], however, glycosyl azides [48] are the most common precursors. The azides are readily prepared from the corresponding glycosyl halides by reaction with silver azide [49], or sodium azide under phase transfer catalysis. The latter has been reported to give higher yields [50]. The glycosyl azide can then be catalytically reduced to the glycosylamine by the use of Pd/C [51], Lindlar's catalyst [52], PtO<sub>2</sub>, Raney Ni [53] or by employing propane dithiol in the presence of triethylamine and methanol [54].

The first reported synthesis of an *N*-glycoamino acid was described by Marks and Nueberger [55] in pursuit of evidence for the proposed carbohydrate–protein

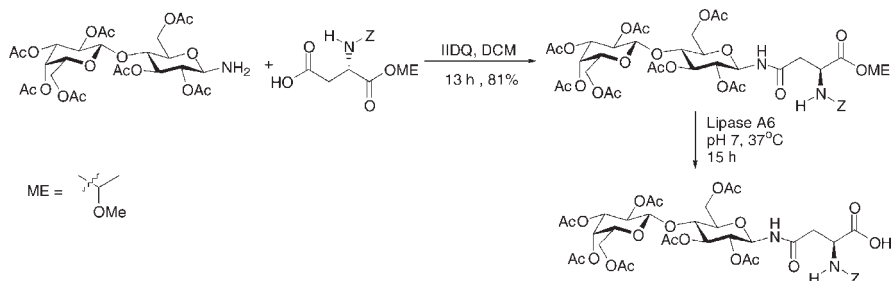


Scheme 10.10

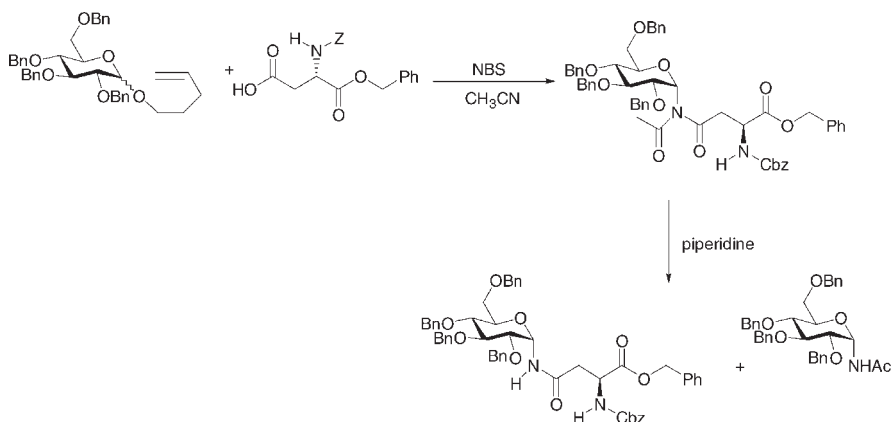
linkage in egg albumin (Scheme 10.10) [56]. Coupling of glycosyl amine with aspartic acid derivatives afforded the  $\beta$ -anomer in 66% yield, with no  $\alpha$ -anomer being observed. Bolton and Jeanloz [57] also reported this reaction employing either dicyclohexylcarbodiimide or 2-ethyl-5-phenylisoxazolium-3'-sulfonate, (WRK, Woodward's reagent K), but isolated only a small amount of the product. Alternatively, reaction of the glycosylamine with 1-benzyl-*N*-carbobenzyloxyl-L-aspartyl chloride in the presence of base gave the desired product in only 28% yield. Contrary to this, the condensation of a glycosylamine with a suitably protected carboxylic acid of Asn has proved a reliable and popular approach [58]. The acylating reagents applicable for peptide bond formation such as dicyclohexylcarbodiimide (DCC), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), WRK, WSC, HOBt and 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ) can also be used for this coupling reaction.

Kunz *et al.* have utilized protecting groups that can be hydrolyzed via lipase-catalysed cleavage, finding that the 2-methoxy ethyl (ME) esters proved most useful for protection of the carboxylic acid moiety in glycopeptides. Cleavage of this group can be carried out selectively by lipase under mild conditions (pH 7.0 at 37 °C), without affecting the peptide bonds or other ester protecting groups [59]. Out of the 34 lipases used, lipase N (Amano) from *Rhizopus niveus*, lipase M (Amano) from *Mucor javanicus* and lipase A6 (Amano) from *Aspergillus niger*, afforded best results. (Scheme 10.11). Sugai *et al.* [60] have also reported the enzyme-catalyzed hydrolysis of an  $\alpha$ -ester *N*-glycoside containing an unprotected sugar residue in a short, high yielding synthesis of GlcNAc-Cbz-Asn.

Another alternative protecting group for the amino acid is 1,3-dithian-2-ylmethyl (Dim) which was again employed by Kunz in the protection of the acid moiety of



Scheme 10.11

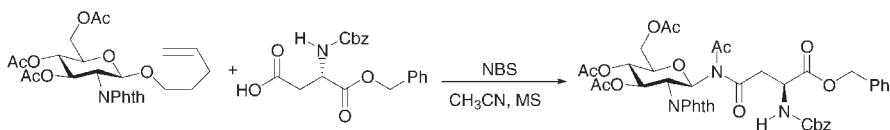


Scheme 10.12

the *N*-glycopeptide Boc-aspartic acid  $\alpha$ -Dim esters [61]. Again the Dim group can be selectively cleaved under mild conditions using hydrogen peroxide and ammonium molybdate. The resulting glycoside is base sensitive, consequently maintaining the reaction at pH 8 results in the glycosyl amino acid being isolated with quantitative removal of the *C*-terminal protecting group.

*Via n-pentyl glycosides.* Fraser-Reid has reported new methodology [62] that allows the direct coupling of a peptidyl residue with a pent-4-enyl glycosyl donor to stereoselectively afford an  $\alpha$ -linked glycopyranosylamide [63] (Scheme 10.12) [64]. Selective *N*-deacetylation was achieved by exposure of the *N*-linked glycoamino acid to piperidine in anhydrous DMF affording the  $\alpha$ -linked asparagine derivative in 89% yield. The acetamido compound was also obtained in 3.5% yield. The two products could be isolated employing flash-column chromatography. Removal of the protecting groups via catalytic hydrogenolysis with 10% Pd/C affords  $\alpha$ -Glc-(1-Asn). A similar approach, using phenyl thioglycosides, has also been reported by Sasaki *et al.* in pursuit of a stereocontrolled synthesis of the nephritogenoside core structure [65]. The reaction is believed to proceed through the same mechanism, via the  $\alpha$ -acetonitrilium ion, and affords the  $\alpha$ -imide in an improved 85% yield.

This alternative approach was also applied for the synthesis of a protected *N*- $\beta$ -linked chitobiose–asparagine conjugate [66] (Scheme 10.13). Trapping the intermediate with the acetonitrile solvent then affords the  $\alpha$ -nitrilium ion.



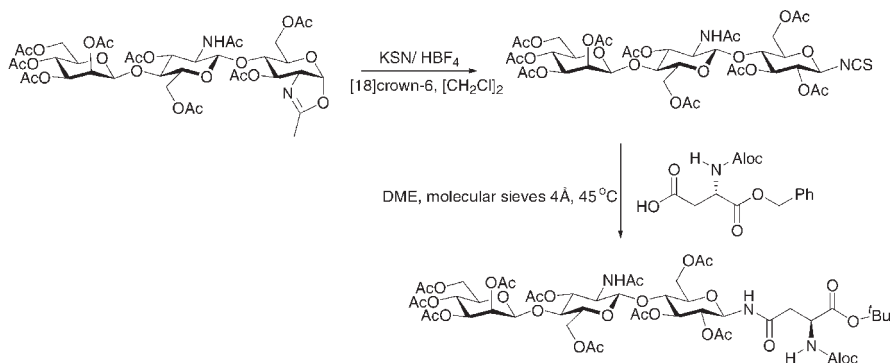
Scheme 10.13

Addition of a carboxylic acid thus afforded the imide via the rearrangement of an imino anhydride intermediate. Deprotection of the acetyl group to access the *N*-glycosylamino acid can be easily obtained by reaction with piperidine in DMF.

*Via anomeric isothiocyanates.* Kunz and Gunther employed the anomeric isothiocyanates in the synthesis of a  $\beta$ -linked chitobiosyl–asparagine conjugate. The key step for the formation of the *N*-glycosidic bond involved coupling of aspartic acid with a glycosyl isothiocyanate (Scheme 10.14) [67].  $\beta$ -Mannosyl–chitobiosyl–asparagine was obtained in 78% yield by the reaction of isothiocyanide with  $\alpha$ -*t*-butyl *N*-allyloxycarbonyl L-asparaginate under anhydrous conditions. Formation of the isothiocyanide was achieved by reacting oxazoline with potassium rhodanide (potassium thiocyanate/HBF<sub>4</sub>) employing an 18-crown-6 catalyst.

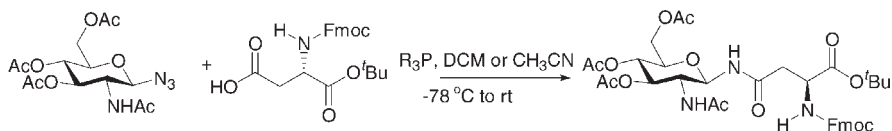
*Via glycosyl azides.* The formation of an amide bond by reaction of an azide and a carboxylic acid in the presence of a tertiary phosphine is a well established reaction [68]. Maunier *et al.* [69] have successfully used this method in the synthesis of a glycosyl amide via the reaction of a glycosyl azide and acid chloride in the presence of a trialkylphosphine. In addition to this, Inazu and Kobayashi have also employed this approach in the synthesis of *N*-glycosylated-L-asparagine acid  $\alpha$ -monoester [70]. The reaction of a glycosyl azide and *N*-protected-aspartic acid  $\alpha$ -monoesters in the presence of triethylphosphine allows entry to *N*-glycosylated-L-asparagine acid  $\alpha$ -monoesters, which are valuable precursors for elaboration into *N*-glycopeptides.

Optimization of the reaction by the use of different trialkylphosphines at low temperatures has been used to afford glycosyl asparagine and glycosyl glutamine analogues (Scheme 10.15) [71]. The importance of the reaction conditions is illustrated by the observation that no product was isolated on use of the triphenyl- and trimethoxyphosphines, however, best results were obtained when triethylphosphine was utilized. The choice of solvent is also important with dimethylformamide and methanol proving to be poor solvents, whereas DCM and acetonitrile afforded the best results.



Scheme 10.14



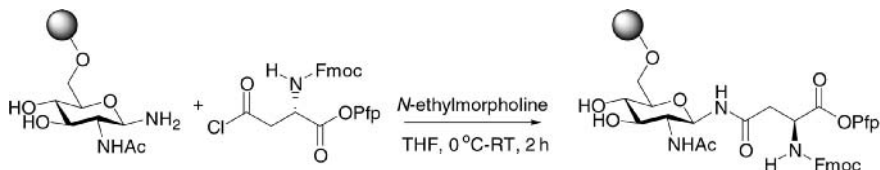


Scheme 10.15

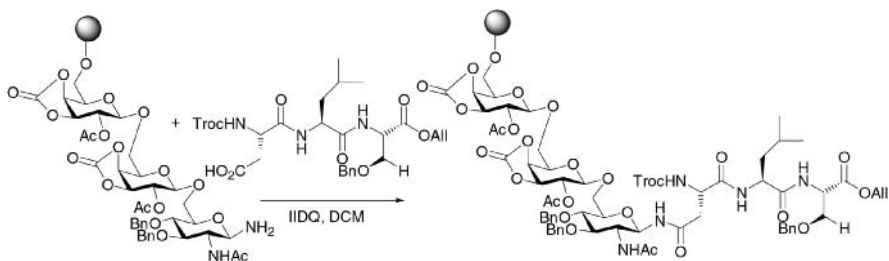
#### 10.4 SOLID PHASE SYNTHESIS OF *N*- AND *O*-GLYCOSYLAMINO ACIDS AND *N*- AND *O*-GLYCOPEPTIDES

Solid phase synthesis of unprotected *N*-glycopeptides [72] employing a trityl chloride linker on a polystyrene resin has been reported (Scheme 10.16). The resin is easily added to the Fmoc protected glycosyl amine in the presence of pyridine. The resin was cleaved with 2% trifluoroacetic acid to afford a glycosylated asparagine building block in 70% yield.

Solid phase synthesis has also been successfully applied for the preparation of glycopeptides via stepwise approaches, by coupling a glycosyl amine with a suitably protected Asp derivative. Problems can sometimes occur as the sugar is introduced at an early stage and some of the *O*-glycosidic bonds may be incompatible with the peptide synthesis conditions. Consequently, the convergent approach has often found more use [73]. An example of a convergent approach towards *N*-glycopeptides has been described by Danishefsky [74] (Scheme 10.17). The tripeptide was prepared using standard solution-phase peptide chemistry. The resin was removed by employing hydrofluoric acid in pyridine.



Scheme 10.16



Scheme 10.17

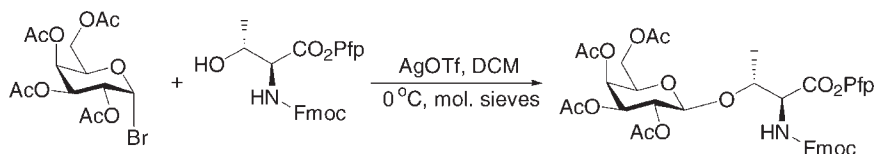
## 10.5 CONCLUSION

The development of selective synthetic methodologies has allowed the field of glycopeptide chemistry to constantly expand and diversify, providing access to highly complex structures on preparative scales. Biological investigations into the roles of glycopeptides demand better and cleaner synthetic methods. Future efforts in glycopeptide chemistry will particularly focus on improved synthesis of glycosylation building blocks either in solution or solid phase using chemical or enzymic methods [75]. Indeed, new *S*-linked glycosides with enhanced stability to chemical and enzymic degradation have recently been prepared by solid phase synthesis [76].

## 10.6 EXPERIMENTAL PROCEDURES

### Method 1

The preparation of *N*-(9-fluorenylmethoxycarbonyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranosyl)-L-threonine pentafluorophenyl ester [14].



### Notes and discussion

This high yielding Koenigs–Knorr glycosylation reaction can be easily adapted to incorporate different donors and acceptors. Thus mannose [35], glucose [77] and glucosamine donors [41], and serine and threonine acceptors have been utilized. Although the reaction is usually carried out in pure dichloromethane, toluene can be used as a co-solvent. The example here is carried out at 0 °C but temperatures from  $-30$  to  $-40$  °C have been used to avoid by-product formation. Naturally these lower temperatures require longer reaction times (7–8 h at  $-40$  °C).

A base such as pyridine, 2,4,6-collidine,  $\text{Pr}_2\text{NEt}$  or *N*-methylmorpholine is added to neutralize the solution before work up [78]. Deprotection of the product can be achieved using morpholine to remove the Fmoc protecting group and hydrazine hydrate or sodium methoxide to remove the acetate groups.

### Materials

2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-galactopyranosyl bromide [79]  
(1.10 g, 2.68 mmol)

assume toxic

Fmoc-Thr-Pfp [80] (0.833 g, 1.64 mmol)	assume toxic
Silver triflate (0.64 g, 2.5 mmol)	irritant/light sensitive
Dichloromethane (25 ml)	harmful
Dry toluene	harmful
Molecular sieves 3 or 4 Å (predried in oven)	dust hazard
Aqueous NaHCO <sub>3</sub>	irritant

### *Equipment*

Round bottomed flask (50 ml) with stirrer bar  
 Magnetic stirrer  
 Syringe  
 Nitrogen balloon/manifold  
 Ice bath  
 Suction filter  
 Separating funnel and conical flasks  
 Rotary evaporator  
 Column chromatography equipment

### *Special precautions*

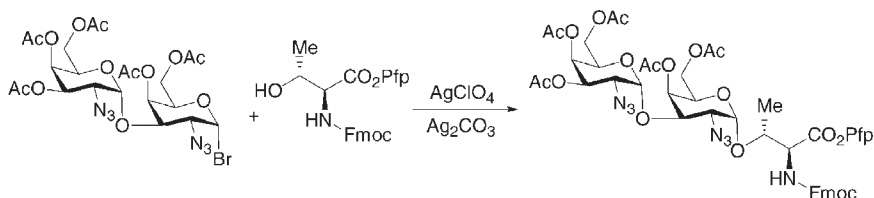
All glassware must be dried prior to use. The operator should wear safety glasses and gloves, and the reaction should be carried out in a fume hood.

Into a dry round bottomed flask equipped with a stirrer bar and rubber septum add the Fmoc-Thr-Pfp [80] (0.833 g, 1.64 mmol) and 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-galactopyranosyl bromide [79] (1.10 g, 2.68 mmol) with powdered molecular sieves (4 Å, 1.1 g) followed by dry dichloromethane (15 ml). Place the mixture under nitrogen (nitrogen balloon) and cool to 0 °C. In a separate dry vial (with rubber septum) dissolve silver triflate (0.64 g, 2.5 mmol) in dry toluene (5 ml). Add the silver triflate solution slowly to the stirred mixture, cover with foil and stir for 1 h. Remove the ice bath and allow the mixture to reach room temperature with stirring for 2 h. Filter off the inorganic impurities and wash the filter funnel with dichloromethane (50 ml). Transfer the mixture to a separating funnel and wash the organic solution with aqueous NaHCO<sub>3</sub> (80 ml). Collect the lower organic layer and add magnesium sulfate. Filter the suspension and remove the solvent under reduced pressure. The crude residue can be purified by flash-column chromatography on silica eluting with heptane–ethyl acetate (3:1) or toluene–ethyl acetate (4:1) to give the product (0.78 g, 57%)  $[\alpha]_D^{25} = -24^\circ$  (*c* 0.30, CDCl<sub>3</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 5.73 (d, *J* = 9.0 Hz, 1H, NH), 5.38 (d, *J* = 2.9 Hz, 1H, H-4), 5.18 (dd, *J* = 7.8, 10.3 Hz, 1H, H-2), 5.04 (dd, *J* = 3.4, 10.5 Hz, 1H, H-3), 4.72 (dd, *J* = 2.5,

9.1 Hz, 1H, H- $\alpha$ ), 4.58 (dd,  $J = 2.7, 6.1$  Hz, 1H, H- $\beta$ ), 4.52 (d,  $J = 7.8$  Hz, 1H, H-1), 4.50 (dd,  $J = 7.2, 10.8$  Hz, 1H, OCOCH<sub>2</sub>CH), 4.43 (dd,  $J = 7.4, 10.5$  Hz, 1H, OCOCH<sub>2</sub>CH), 4.27 (t,  $J = 7.1$  Hz, 1H, OCOCH<sub>2</sub>CH), 4.03 (m, 2H, H-6,6'), 3.89 (t,  $J = 6.4$  Hz, 1H, H-5), 2.17, 2.07, 2.01, and 2.00 (4s, 3H each, Ac), 1.31 (d,  $J = 6.2$  Hz, 3H). <sup>13</sup>C NMR (75 MHz) 170.3, 170.3, 170.1, 169.4, 166.3, 156.5, 143.8, 143.6, 141.3, 127.8, 127.1, 125.1, 120.0, 98.6, 73.7, 70.7, 70.6, 69.0, 67.4, 66.7, 60.8, 58.6, 47.2, 20.8, 20.6, 20.5, 20.4, 16.7.

### Method 2

Preparation of the *N*<sup>α</sup>-Fmoc-*O*-[ $\alpha$ -D-Ac<sub>3</sub>GalN<sub>3</sub>*p*-(1  $\rightarrow$  3)- $\alpha$ -D-Ac<sub>2</sub>GalN<sub>3</sub>*p*]-Thr-OPfp building block [81].



### Notes and discussion

This high yielding, stereoselective glycosylation reaction exclusively affords the  $\alpha$ -anomer of the product. The donor is used in slight excess (1.3 equiv.). When the  $\beta$ -chloro donor is employed, these reactions are not stereospecific although the  $\alpha$ -glycoside does predominate and can usually be separated by chromatography [82]. The acetyl groups used to protect the carbohydrate can be easily removed using a catalytic amount of sodium methoxide in methanol. This is useful in the synthesis of the tumor associated T<sub>N</sub> antigen (GalNAc- $\alpha/\beta \rightarrow$  Ser/Thr). The 2-acetamido deoxy sugar can be used as starting material to allow access to the  $\beta$ -glycoside.

### Materials

$\alpha$ -D-Bromo galactose derivative [79] (265 mg, 0.4 mmol, 1.3 eq.)	assume toxic
Fmoc /Pfp-Threonine [80] (168 mg, 0.33 mol, 1 eq)	assume toxic
Silver carbonate (110 mg, 0.4 mmol)	irritant/light sensitive
Silver perchlorate (11 mg)	corrosive
4 Å molecular sieves	dust hazard
Dry dichloromethane	harmful /irritant

Dry toluene	harmful/highly flammable
Aqueous NaHCO <sub>3</sub>	irritant

### *Equipment*

Round bottomed flask (25 ml) with stirrer bar  
 Magnetic stirrer  
 Syringe and needle × 2  
 Nitrogen balloon/manifold  
 Separating funnel and conical flasks  
 Suction filter  
 Mortar and pestle  
 Rotary evaporator  
 Column chromatography equipment

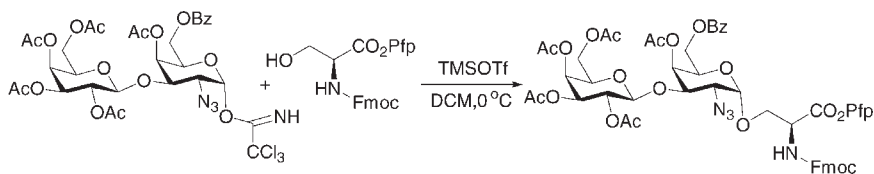
### *Special precautions*

All glassware must be dried prior to use. The operator should wear safety glasses and gloves and the reaction should be carried out in a fume hood.

Dry the 4 Å molecular sieves in an oven overnight and powder in a mortar and pestle. Into a dry round bottomed flask equipped with a stirrer bar, add the protected amino acid (168 mg, 0.33 mol, 1 equiv.), activated molecular sieves (at least 2 equiv. by weight) and silver carbonate (110 mg, 0.4 mmol) and dissolve in dry dichloromethane (2.5 ml) and dry toluene (2.5 ml). Stir the suspension for 1 h at room temperature under nitrogen. To this mixture add silver perchlorate (11 mg) followed by a solution of the glycosyl bromide (265 mg, 0.4 mmol, 1.3 equiv.) dissolved in dry dichloromethane (1.5 ml) and toluene (1.5 ml). Cover the flask in foil to protect from light and stir for 20 h (overnight) at room temperature. Filter the solution to remove the inorganic impurities and wash the filter paper with dichloromethane (10 ml × 2). Transfer the organic layer to a separating funnel and wash with aq. NaHCO<sub>3</sub> and water, dry using Na<sub>2</sub>SO<sub>4</sub> or MgSO<sub>4</sub> then filter and evaporate the solvent under reduced pressure to give a crude residue which is purified by flash-column chromatography on silica gel (20 g) eluting with toluene–ethyl acetate (3:1) to give the product (265 mg, 73%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 1.42 (3H, d, Thr), 1.95–2.19 (15H, 5s, 5 × Ac), 3.68 (1H, dd, *J* = 4.1, 11.2 Hz, H-2'), 3.83 (1H, dd, *J* = 3.6, 10 Hz, H-2), 3.93 (1H, dd, *J* = 6.6, 10.2 Hz, H-6<sup>a</sup>), 4.05 (1H, dd, *J* = 7.1, 11.2 Hz, H-6<sup>a</sup>), 4.14 (1H, dd, *J* = 3.6 Hz, H-3), 4.16 (1H, dd, *J* = 7.1, 11.2 Hz, H-6<sup>b</sup>), 4.18–4.28 (4H, m, Fmoc CH, H-5, H-5', H-6<sup>b</sup>), 4.47 (1H, dd, *J* = 7.1, 10.7 Hz, Fmoc CH<sub>2</sub>), 4.52 (1H, dd, *J* = 7.2 Hz, Fmoc CH<sub>2</sub>), 4.58 (1H, dd, *J* = 6.1, 2.0 Hz, Thr β-H), 4.79 (1H, dd, Thr α-H), 5.19 (1H, d, H-1), 5.32 (1H, d, H-1'), 5.37 (1H, dd, *J* = 3.05 Hz, H-3), 5.48 (1H, dd, *J* = 1.0 Hz, H-4'), 5.54 (1H, dd, *J* = 1.0 Hz, H-4), 6.66 (1H, d, *J* = 9.6 Hz, ThrNH), 7.12–7.78 (8H, m, ArH).

### Method 3

Preparation of *N*-Fmoc-*O*-[2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranosyl-(1  $\rightarrow$  3)-4-*O*-acetyl-2-azido-6-*O*-benzoyl-2-deoxy- $\alpha$ -D-galactopyranosyl]-L-serine Pfp ester.



### Notes and discussion

This method allows the preparation of the core T-antigen building block using a glycosyl imidate as donor [27]. This transformation can also be achieved using boron trifluoride etherate activation of  $\beta$ -Gal(1  $\rightarrow$  3)- $\alpha$ -GalNAc-imidate and reaction with suitably protected serine or threonine acceptors [29].

The *N*-Fmoc protected pentafluorophenyl serine was prepared according to the method of Schön [80]. The serine glycopeptide was obtained in 48% yield and the threonine analogue obtained in 68% yield. The reaction with serine gave a single anomer but with threonine, a 3:1 mixture of  $\alpha/\beta$  anomers formed. Notably the product was highly susceptible to hydrolytic cleavage and decomposed on silica gel, hence, florisil was employed for the purification stage.

### Materials

$\beta$ -D-Ac <sub>4</sub> Gal(1 $\rightarrow$ 3)- $\alpha$ -D-6-OBz,4-OAcGalN <sub>3</sub> -OC(CCl <sub>3</sub> )=NH [83] (0.5 g, 0.6 mmol)	assume toxic
Fmoc/Pfp-Ser [80] (0.4 g, 0.78 mmol)	assume toxic
TMSOTf (6 $\mu$ l, 0.3 mmol)	flammable/ corrosive/moisture sensitive
Dry dichloromethane (4 ml)	harmful/irritant
Pyridine (5 $\mu$ l)	harmful/flammable
Florisil (20 g)	dust hazard
Toluene	harmful /highly flammable
Acetone	flammable

### Equipment

Round bottomed flask (10 ml) with stirrer bar  
 Magnetic stirrer  
 Syringe and microsyringe  
 Nitrogen balloon/manifold  
 Rotary evaporator  
 Column chromatography equipment

### Special precautions

All glassware must be dried prior to use. The operator should wear safety glasses and gloves and the reaction should be carried out in a fume hood.

Into a dry round bottomed flask with a stirrer bar and rubber septum, add the glycosyl imidate (0.5 g, 0.6 mmol) and the Fmoc/Pfp protected serine (0.4 g, 0.78 mmol). Add dry dichloromethane (3 ml) with stirring, and cool to 0 °C under a nitrogen atmosphere (nitrogen balloon). In another dry flask or vial fitted with a rubber septum dilute trimethylsilyl trifluoromethanesulfonate (6  $\mu$ l, 0.3 mmol) in dry dichloromethane (0.5 ml). Add the TMSOTf solution slowly to the mixture and stir for 2 h. Monitor the reaction by TLC (product  $R_f$  = 0.58 in toluene–acetone 3:1) and when complete neutralize the mixture by addition of pyridine (5  $\mu$ l) and remove the solvent under reduced pressure. Purify the crude residue by flash-column chromatography on florisil eluting with toluene–acetone (12:1) to give the glycopeptide product (694 mg, 42%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 250 MHz) 1.96–2.15 (5s, 15H, 5Ac), 3.71 (dd, 1H,  $J$  = 2.7 Hz, 10.7, H-3), 3.84 (m, 1H, H-5), 4.04–4.39 (m, 11H), 4.65 (d, 1H,  $J$  = 7.7 Hz, H-1'), 4.90 (m, 1H, Ser- $\alpha$ -CH), 4.93 (dd, 1H,  $J$  = 10.5, 3.1 Hz, H-3'), 4.99 (d, 1H,  $J$  = 3.2 Hz, H-1), 5.17 (dd, 1H, H-2'), 5.34 (d, 1H, H-4), 5.56 (d, 1H, H-4'), 5.96 (d, 1H,  $J$  = 8.7 Hz, NH), 7.25–8.04 (m, 13H, Fmoc, Bz).

### Method 4

Preparation of the  $\text{N}^4$ -(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)- $\text{N}^2$ -(*tert*-butyloxycarbonyl) asparagine 1,3-dithian-2-ylmethyl ester [61].



*Notes and discussion*

The Boc-aspartic acid Dim ester starting material can be prepared in two steps from *N*-(*tert*-butyloxycarbonyl) aspartic acid  $\beta$ -phenacyl ester [61]. This process involves the stereocontrolled reaction of *N*-(Boc)-*N*-glycosylated-L-asparagine (Dim) ester derivative with a glycosyl amine to only afford the  $\beta$ -anomer. Isolation can be easily achieved by recrystallization from DCM/petroleum ether. For further elaboration of the peptide chain the Dim group can be readily cleaved under mild conditions, leaving the newly formed *N*-glycosidic bond and other protecting groups intact.

*Materials*

2-Acetylamido-3,4,6-tri- <i>O</i> -acetyl-2-deoxy- $\beta$ -glucopyranosylamine (0.56 g, 1.6 mmol)	assume toxic
<i>N</i> -Butyloxycarbonyl-L-aspartic acid $\alpha$ -(1,3-dithian-2-ylmethyl) ester (0.5 g, 1.36 mmol)	assume toxic
2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (0.047 g, 1.9 mmol)	harmful
Dichloromethane	harmful /irritant
Aq. NaHCO <sub>3</sub>	irritant
0.1 M HCl	corrosive /harmful
MgSO <sub>4</sub>	irritant

*Equipment*

Round bottomed flask (100 ml) with stirrer bar  
 Magnetic stirrer  
 Syringe and needles  $\times$  2  
 Nitrogen balloon/manifold  
 Separating funnel and conical flasks  
 Filter and filter paper  
 Rotary evaporator  
 Column chromatography equipment

*Special precautions*

All glassware must be dried prior to use. The operator should wear safety glasses and gloves and the reaction should be carried out in a fume hood.

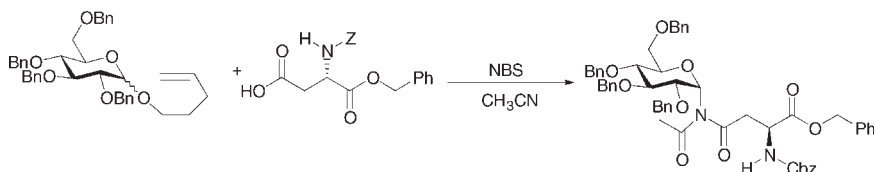
Add the protected amino acid (0.5 g, 1.36 mmol) and the glycosyl amine (0.56 g, 1.6 mmol) into a dry round bottomed flask with a stirrer bar and dissolve in



dry dichloromethane (10 ml). To this mixture add EEDQ (0.047 g, 1.9 mmol) and stir for 18 h at room temperature under nitrogen. Dilute the reaction with dichloromethane (20 ml) and transfer the organic layer to a separating funnel and wash with hydrochloric acid (10 ml), aq.  $\text{NaHCO}_3$  (10 ml) and  $\text{H}_2\text{O}$  (10 ml), then dry using  $\text{Na}_2\text{SO}_4$  or  $\text{MgSO}_4$  and evaporate the solvent under reduced pressure to give the crude product. This can be recrystallized from DCM/petroleum ether to give the product as a white solid (0.7 g, 74%), mp 146–148 °C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 90 MHz) 7.35 (1H, d,  $J = 8.5$  Hz, NH urethane), 6.6 (1H, d  $J = 8.8$  Hz, NH), 5.7 (1H d,  $J = 8.7$  Hz, NH, urethane), 3.0–2.7 (6H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ , and  $\beta$ - $\text{CH}_2\text{Asn}$ ), 2.09, 2.06, 2.04 (9H, s,  $3 \times \text{CH}_3\text{CO}_2$ ), 1.98 (3H, s,  $\text{CH}_3\text{COCH}$ ), 1.43 (9H, s,  $(\text{CH}_3)_2\text{C}$ ).

### Method 5

Preparation of *N*-acetyl-2,3,4,6-tetra-*O*-benzyl-*N*-benzyloxycarbonyl-L- $\beta$ -aspartyl- $\alpha$ -D-glycopyranosylamine [64].



### Notes and discussion

This reaction offers a stereoselective synthesis of  $\alpha$ -linked glucosyl asparagine derivatives from both anomers of pent-4-enyl D-glycopyranoside. The pent-4-enyl 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -/ $\beta$ -D-glycopyranoside starting material for this reaction can be prepared easily in two steps from  $\alpha$ -/ $\beta$ -glycopyranose [84]. For selective *N*-deacetylation of the product best results are obtained with piperidine which affords the  $\alpha$ -linked asparagine derivate in 89% yield, the yield of the acetamido side product was reduced to 3.5%. The reaction time is also shortened to 5.75 h (when diethylamine was employed, the reaction was complete after 39 h with worse yields). Note that no reaction occurs when diisopropylamine is employed.

### Materials

Pent-4-enyl 2,3,4,6-tetra- <i>O</i> -benzyl- $\alpha$ -/ $\beta$ -D-glycopyranoside (337 mg, 0.55 mmol)	assume toxic
$\alpha$ -Benzyl- <i>N</i> -benzyloxycarbonyl-L-aspartic acid (215 mg, 0.60 mmol)	assume toxic

Dry acetonitrile	toxic
<i>N</i> -Bromosuccinimide (156 mg, 0.88 mmol)	irritant
Aq. 10% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	irritant
Chloroform	harmful/irritant
MgSO <sub>4</sub>	irritant

### Equipment

Round bottomed flask (100 ml) with stirrer bar  
Magnetic stirrer  
Syringe and needles × 2  
Nitrogen balloon /manifold  
Separating funnel  
Suction filter  
Rotary evaporator  
Column chromatography equipment

### Special precautions

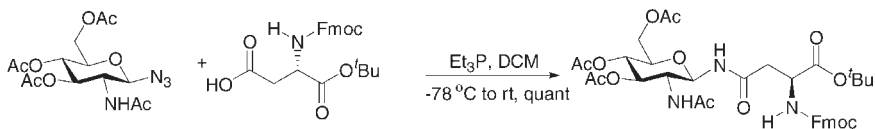
All glassware must be dried prior to use. The operator should wear safety glasses and gloves and the reaction should be carried out in a fume hood.

*N*-Bromosuccinimide should be recrystallized from hot water (10 equiv. by weight) and dried *in vacuo* over phosphorus pentoxide before use.

Into a dry round bottomed flask with a stirrer bar add pent-4-enyl 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -/ $\beta$ -D-glucopyranoside (337 mg, 0.55 mmol), and dissolve in dry acetonitrile (11 ml) then add the protected amino acid (215 mg, 0.60 mmol) followed by *N*-bromosuccinimide (156 mg, 0.88 mmol). Cover the flask in foil to protect from light and stir at room temperature under argon for 2.75 h. To the light green solution add aqueous 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2 ml). Evaporate the bulk of the organic solvent under reduced pressure. To the resulting residue add water (50 ml) and chloroform (50 ml) and transfer into a separating funnel. Extract the aqueous layer with chloroform (4 × 50 ml) and combine the organic layers then dry using Na<sub>2</sub>SO<sub>4</sub> or MgSO<sub>4</sub> and evaporate the solvent under reduced pressure to give a crude product which is purified by flash-column chromatography on silica gel eluting with chloroform–acetone (80:1 then 40:1) to give the product as an oil (271 mg, 53%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) 7.28–7.18 (30H, m, 6 × Ph), 5.90 (1H, d, *J* = 8.4 Hz, NHCOBn), 5.59 (1H, d, *J* = 5.7 Hz, H-1), 5.20–5.02 (4H, m, 2 × CO<sub>2</sub>CH<sub>2</sub>Ph), 4.71–4.39 (9H, m, 4 × OCH<sub>2</sub>Ph and COCH<sub>2</sub>CH), 4.17–4.14 (1H, m, H-5), 4.04 (1H, t, *J* = 6.8 Hz, H-3), 3.82 (1H, t, *J* = 6.1 Hz, H-2), 3.72–3.64 (2H, m, H-4, 6a), 3.56 (1H, dd, *J* = 10.5 Hz, 1.9, H-6b), 3.34 (1H, dd, *J* = 17.8, 4.2 Hz, COCH<sub>A</sub>-H<sub>B</sub>CH), 3.26 (1H, dd *J* = 17.8, 5.5 Hz, COCH<sub>A</sub>H<sub>B</sub>CH), 2.12 (3H, s, NHAc).

### Method 6

Preparation of  $N^{\omega}$ -(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glycopynosyl) $N^{\alpha}$ -(9-fluorenylmethyloxycarbonyl)-L-asparagine *tert*-butyl ester [71].



### Notes and discussion

The stereoselectivity of this reaction is dependent on the order of addition of the reagents. Final addition of Fmoc-Asp-OBn to the reaction mixture stops formation of the  $N$ - $\beta$ -glycosylated-L-asparagine derivative product being obtained. Best yields are achieved when the glycosyl azide displays a 2-acetamido group, in the absence of this functionality worse yields are obtained. The reaction only works in DCM or acetonitrile and DCM gave better yields. Performing the reaction at lower temperatures ( $-78^{\circ}\text{C}$ ) affords higher yields of the required product as generation of the glycosyl phosphazene and nitrogen elimination is hindered, allowing successful amide bond formation.

### Materials

2-Acetyl-amido-3,4,6-tri- <i>O</i> -acetyl-2-deoxy- $\beta$ -glucopyranosyl azide (18.94 g, 51 mmol)	assume toxic
$N^{\alpha}$ -(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid $\alpha$ - <i>tert</i> -butyl ester (17.42 g, 42 mmol)	assume toxic
Triethylphosphine (5.00 g, 42 mmol)	harmful /toxic
Dry ice/acetone	flammable
Dichloromethane	harmful/irritant
Diethyl ether	highly flammable
5% (citric acid aq.)	irritant
Brine	
Sodium sulfate	irritant

### Equipment

Round bottomed flask (500 ml) with stirrer bar  
 Magnetic stirrer  
 Syringe and needles  $\times 2$

– 78 °C cooling bath  
Nitrogen balloon/manifold  
Separating funnel  
Suction filter  
Rotary evaporator  
Column chromatography equipment

### *Special precautions*

All glassware must be dried prior to use. The operator should wear safety glasses and gloves and the reaction should be carried out in a fume hood.

Into a dry round bottomed flask equipped with a stirrer bar add the protected amino acid (17.42 g, 42 mmol) followed by the glycosyl azide (18.94 g, 51 mmol) and dissolve in dry dichloromethane (200 ml) at – 78 °C under nitrogen. To this mixture add triethylphosphine (5.00 g, 42 mmol) and stir at – 78 °C under nitrogen overnight. A precipitate will form in the reaction mixture due to poor product solubility.

Filter the resulting precipitate to obtain the product and wash with dichloromethane (50 ml  $\times$  1) and with diethyl ether (50 ml  $\times$  3) to give the product as a white powder (24.97 g, 80%). Collect the filtrate and evaporate under reduced pressure to give a residue. Dissolve the residue in dichloromethane (100 ml) and transfer the organic layer to a separating funnel and wash with 5% citric acid, followed by water, and brine, then dry using Na<sub>2</sub>SO<sub>4</sub> or MgSO<sub>4</sub> and evaporate the solvent under reduced pressure to give a residue. Wash the residue with diethyl ether (50 ml  $\times$  3) to give a second crop of the product (7.06 g, 20%), quantitative yield overall. Mp 213–214 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 1.44 (9H, s, *t*-Bu), 1.96, (3H, s, NAc), 2.04, 2.05, 2.07 (9H, s, 3  $\times$  OAc), 2.68–2.86 (2H, m, Asn- $\beta$ -CH<sub>2</sub>), 3.74–3.77 (1H, m, glc-5-H), 4.04–4.44 (6H, m, 2,6-H, Fmoc-CH<sub>2</sub>, Fmoc-CH), 4.52 (1H, m, Asn- $\alpha$ -CH), 5.05–5.15 (3H, m, 1,3,4-H), 5.98 (1H, d, *J* = 8.8 Hz, Asn- $\alpha$ -NH), 6.17 (1H, d, *J* = 8.3 Hz, 2-NH), 7.20 (2H, pseudo-*t*, Fmoc-Ar), 7.60 (2H, d, *J* = 7.3 Hz, Fmoc-Ar), 7.75 (2H, d, *J* = 7.8 Hz, Fmoc-Ar).

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## The Synthesis of C-linked Glycosides

Paul Meo and Helen M.I. Osborn

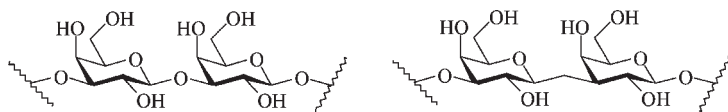
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### 11.1 INTRODUCTION

It is now well established that oligosaccharides and glycoconjugates are involved in a multitude of biological processes including cell recognition, cell differentiation and cell adhesion. In addition, a number of protein–carbohydrate interactions mediate critical biological processes such as cell signaling in growth and differentiation, and fertilization [1]. Whilst the above processes are generally beneficial, carbohydrates have also been identified to play major roles in a number of detrimental processes, such as inflammation, viral and bacterial infections, and tumour metastasis. For example, the adhesion properties of cancer cells are believed to result from interactions between specific lectins (carbohydrate binding proteins) of the host and disease associated glycoproteins on the tumour cell surface membrane [2]. This suggests that inhibition of the synthesis of the disease associated carbohydrates, or blocking of the lectin/disease glycoprotein interactions, may hold the key to the successful treatment of some diseases [3]. Major achievements in the development of carbohydrate based therapeutics include the synthesis of a pentasaccharide heparin fragment, which displays selective antithrombic activity [4], and the design and synthesis of derivatives of sialyl Lewis X, that have proved of interest as potential anti-inflammatory agents [3c]. However, the glycosidic bonds connecting individual saccharide units within carbohydrate based therapeutics are unstable to glycosidase enzymes *in vivo* and agents of enhanced stability must be developed if the therapeutic potential of carbohydrate based drugs is to be fully exploited. To overcome this limitation, a considerable amount of research has focused on the generation of C-glycosides, molecules that are analogues of regular saccharides but in which the interglycosidic oxygen atom has been replaced by a methylene group (Figure 11.1).

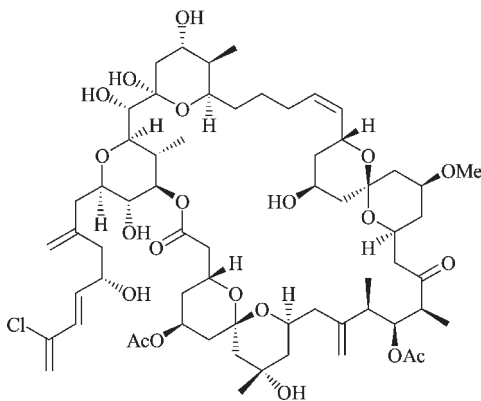




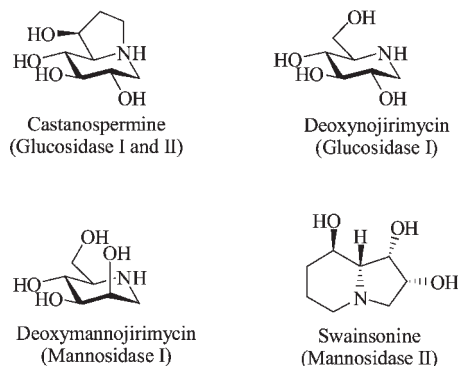
**Figure 11.1** O- and C-glycosidic bonds.

Such compounds can be considered as chemically inert isosteres of natural *O*- and *N*-glycosides that are stable to glycosidase enzymes *in vivo*. A number of natural *C*-linked nucleosides with potent pharmacological properties have also been isolated. With the subsequent discovery of several natural products [5], such as Spongistatin 1 (Figure 11.2) [6], which exhibit structural motifs analogous to *C*-glycosides, the ubiquitous nature of this new functionality and its therapeutic potential have been realised.

Synthetically prepared *C*-glycosides have so far proved of use for probing stereoelectronic effects which may control the conformation of oligosaccharides [7] and have also been shown to prevent viral and bacterial attachment to lectins in adhesion assays, to the same extent as *O*-glycosides [8]. *C*-Glycosides have also proved of use for inhibiting some carbohydrate processing enzymes [9]: oligosaccharides and glycoconjugates are synthesized via complicated and yet highly efficient enzyme mediated routes and the structure of the final glycoconjugate is governed by a series of enzymes [10]. Alterations in the regulation of these enzymes are believed to be responsible for the synthesis of disease associated carbohydrates. For example, the up-regulation of certain glycosyltransferases (GlcNAc-transferase-V) and the down-regulation of others during glycoprotein synthesis see the development of highly branched glycoproteins on cancer cells that promote metastasis. Inhibition of specific steps of this processing pathway offers an exciting opportunity to synthesise different carbohydrates to those formed in diseased pathways. Therefore inhibitors of glycosidase and glycosyltransferase enzymes are currently receiving attention as new pharmaceutical agents. A number



**Figure 11.2** Spongistatin 1.



**Figure 11.3** Naturally occurring glycosidase inhibitors.

of natural inhibitors of the processing enzymes have been isolated and these have provided a greater understanding of the roles of each individual enzyme within carbohydrate synthesis (Figure 11.3) [11].

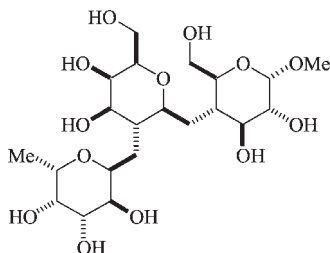
Moreover, a number of enzyme inhibitors have proved of therapeutic potential for the treatment of diseases such as influenza and AIDS. This is because animal viruses contain an external viral envelope, which is often an area of high glycoprotein concentration. These glycoproteins are essential to the life of the virus, providing, in most cases, the means to infect the host and initiate proliferation. Although the precise role for these extracellular glycoproteins in the binding process is still unclear, it is known that interference in their biosynthetic pathways affects viral infectivity [12].

However, biological studies have also shown that the natural inhibitors highlighted in Figure 11.3 exert their effects on a range of glycosidase enzymes and are therefore of limited use as medicinal drugs [13]. Consequently more specific inhibitors are currently being sought that can be engineered to more accurately resemble the substrates that they are mimicking and therefore provide a more specific effect. Early tests on *C*-glycosides have provided evidence for their potential to act as oligosaccharide processing inhibitors [9].

The remainder of this chapter covers the area of *C*-glycoside and *C*-disaccharide synthesis and highlights methodology that has proved invaluable for the synthesis of these targets. It should be noted that whilst the methods described below concentrate on the synthesis of *C*-glycosides and *C*-disaccharides, methodology has also been developed to allow the synthesis of higher *C*-saccharides such as the bis-carba-analogue of a blood group antigenic determinant which is a *C,C*-trisaccharide (Figure 11.4) [14].

## 11.2 SYNTHETIC METHODS

The area of *C*-linked glycoside synthesis has been extensively reviewed over the last 15 years [15]. The following methods highlight the range of chemistry available for



**Figure 11.4** C,C-Trisaccharide analogue of type II O (H) blood group determinant.

the synthesis of C-glycosides, and the ability to perform such reactions in a stereoselective manner.

### 11.2.1 Use of the anomeric centre as a nucleophilic species

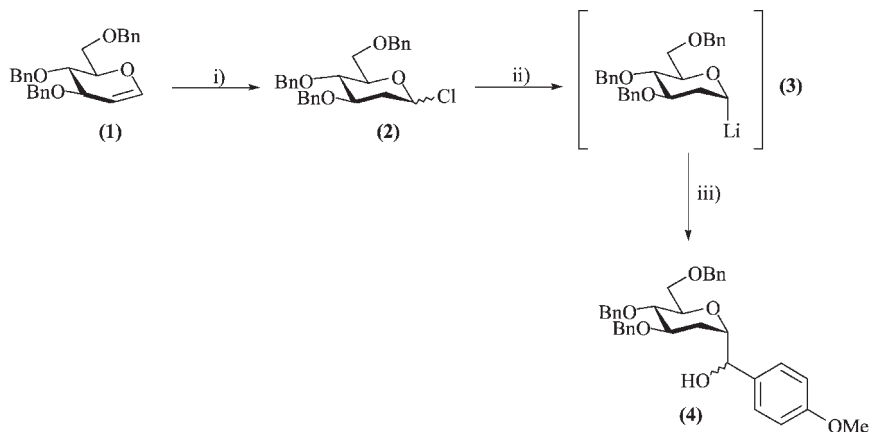
The anomeric centre is, by virtue of the ring oxygen an electrophilic species, and is thus employed as such in numerous *O*-glycosidation strategies. However, the C-1 carbon can actually be converted to a nucleophilic centre, providing a powerful tool for C-glycoside synthesis.

#### 11.2.1.1 Reductive lithiation

The reductive lithiation method allows generation of C-glycosides in good yields with good stereocontrol. Cohen first demonstrated the ability to generate non-stabilised  $\alpha$ -lithioethers by reductive lithiation of  $\alpha$ -(phenylthio)ethers with lithium 1-(dimethylamino) naphthalenide (LDMAN) or lithium naphthalenide (LN) [16]. This method was first applied to carbohydrate chemistry by Sinaÿ. Hydrochlorination of tri-*O*-benzyl-D-glucal (**1**) with HCl produced the 2-deoxy-D-glucopyranosyl chloride (**2**). Reductive lithiation via a two-step single electron transfer mechanism with LN generates the  $\alpha$ -organolithium species (**3**) in good yield. The subsequent reaction of **3** with a series of electrophiles e.g. *p*-MeOC<sub>6</sub>H<sub>4</sub>CHO at  $-78^\circ\text{C}$  produced the  $\alpha$ -C-linked glycoside (**4**) in 65% yield and with excellent stereoselectivity (Scheme 11.1) [17].

The stereoselectivity can be explained by the preference of the anomeric radicals to adopt the more stable axial orientation [18]. Interactions with the non-bonding electron pair of the ring oxygen help to stabilise the radical. However, through careful selection of the sugar protecting groups [19] and a temperature increase to  $-20^\circ\text{C}$ , it is possible to promote isomerisation about the anomeric position generating the  $\beta$ -organolithium intermediate. This, in turn, allows access to  $\beta$ -C-glycosides.

Further methods involving reductive lithiation exploit anomeric sulfones which also lead to the formation of  $\beta$ -C-glycosides [20]. Since C-1-lithiation of carbohydrates traditionally results in 1,2- $\beta$ -elimination, to afford the corresponding

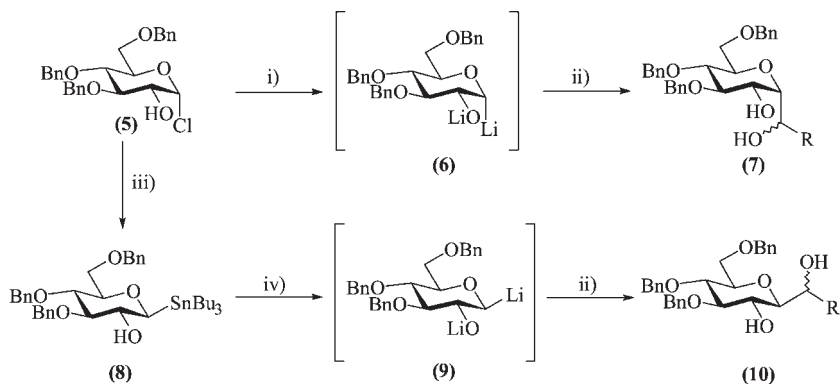


i) HCl, 0°C; ii) LN (2 eq.), -78°C; iii)  $p\text{-MeOC}_6\text{H}_4\text{CHO}$

**Scheme 11.1**

glycal, this method generally allows access to 2-deoxy  $C$ -glycosides. Kessler adapted this method to tolerate incorporation of a heteroatom at C-2 [17, 21]. For example, the addition of  $n\text{-BuLi}$  and LN to 3,4,6-tri- $O$ -benzyl- $\alpha$ -D-glucopyranosyl chloride (5) generated the dianion (6) which could react with a range of suitable electrophiles (Table 11.1) to afford the  $\alpha$ -glycoside (7) with excellent  $\alpha$ -stereoselectivity ( $\alpha/\beta$  20:1) (Scheme 11.2).

The method of Kessler can also be adapted to generate  $\beta$ -glycosides. Sinaÿ had previously shown that transmetalation of organostannanes to the organolithium species occurred with retention of stereochemistry and that the subsequent reaction with electrophiles was stereoselective [22]. Kessler was able to generate



i)  $\text{BuLi}$ , LN, -100°C; ii)  $\text{RCHO}$ ; iii)  $\text{SOCl}_2$ ,  $\text{Bu}_3\text{SnLi}$ ; iv)  $\text{BuLi}$  (10eq.)

**Scheme 11.2**

Table 11.1

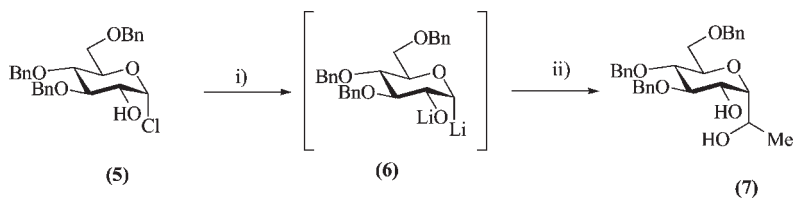
Yields for the $\alpha$ -product (7)		
Electrophile	R	Yield (%)
MeOD	D	75
MeOH	H	82
MeCHO	CH(OH)Me	62
PhCHO	CH(OH)Ph	70
<i>i</i> PrCHO	CH(OH) <i>i</i> Pr	59
HCHO	CH <sub>2</sub> OH	17
MeI	Me	72

the  $\beta$ -stannane (**8**) from the chloride (**5**) ( $\beta/\alpha$  60:1) in good yield. Subsequent transmetallation afforded the  $\beta$ -anomeric anion (**9**) which, upon reaction with a range of electrophiles, allowed access to the  $\beta$ -C-glycoside (**10**) (Scheme 11.2).

In addition, the method can also be adapted to incorporate *N*-acetyl groups at the C-2 position, a functionality which is frequently found in nature [23].

### Method 1

Synthesis of C-glycosides via glycosyl dianions [21].



i) BuLi, LN, -100°C; ii) MeCHO

**Notes and discussion.** This method allows stereoselective synthesis of C-glycosides via glycosyl dianions. Simple lithiation of the free C-2 hydroxyl group of the glucopyranosyl chloride (**5**), with *n*-BuLi, functions both as a means of hydroxyl group protection and efficiently prevents  $\beta$ -elimination. Subsequent reaction with electrophiles allows direct and exclusive entry to 2-hydroxy-substituted  $\alpha$ -C-glucopyranosides. It is reported that best yields are obtained by quick addition of the lithium naphthalenide solution to the anion of 3,4,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranosyl chloride. To avoid undue increase of reaction temperature, use of a precooled lithium naphthalenide solution ( $-78^\circ\text{C}$ ) is also recommended. However, since the solubility of lithium naphthalenide in THF is greatly reduced at this temperature, it is often better to perform the reaction at  $-100^\circ\text{C}$  and add the reagent uncooled. If the transformation yield is low, it has been noted that increased yields can often be attained by the addition of 10% of CuI to the reaction solution before the addition of

the electrophile. No glycal products are formed during this procedure, the dilithio compound is stable at  $-78\text{ }^{\circ}\text{C}$ , and all the reported reactions proceed with retention of configuration at the anomeric centre.

*Materials.*

3,4,6-Tri- <i>O</i> -benzyl- $\alpha$ -D-glucopyranosyl chloride ( <b>5</b> ) [24] (0.15 mol)	treat as harmful
<i>n</i> -Butyl lithium (1.6 M in THF, 1.1 equiv.)	flammable, corrosive, moisture sensitive
Lithium naphthalenide (1 M in THF, 2.2 equiv.)	irritant
Anhydrous THF	flammable, irritant
Acetaldehyde (1.5 equiv.)	treat as harmful
Saturated aqueous ammonium chloride solution	harmful, irritant
Dichloromethane	harmful
Magnesium sulfate	treat as harmful
Hexane, ethyl acetate for chromatography	irritant

*Equipment.*

Round-bottomed flask with rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon or nitrogen  
–  $100\text{ }^{\circ}\text{C}$  cooling bath  
Dry glass syringes and needles  
Separatory funnel and conical flasks  
Filter funnel and filter paper  
Rotary evaporator  
Equipment for chromatography

*Procedure.* All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

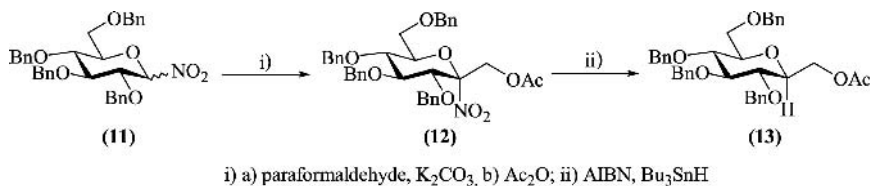
Once the glassware is dry, equip a round bottom flask with a magnetic stirrer bar and rubber septum and flush with dry argon to establish an inert atmosphere. This should be maintained throughout the reaction. Add (**5**) (0.15 mol) and anhydrous THF to the flask and cool to  $-100\text{ }^{\circ}\text{C}$ . Next add *n*-BuLi (1.1 equiv. in THF) and lithium naphthalenide (2.2 equiv. in THF) in quick succession. Then leave the reaction to stir for 15 min. Add acetaldehyde (1.5 equiv.) and leave the reaction for

a further hour maintaining the original temperature, after which time the reaction is warmed to room temperature. Quench the reaction by the addition of a saturated aqueous solution of ammonium chloride, then transfer the solution to a separatory funnel. Extract the aqueous layer three times with dichloromethane. Dry the organic layers with magnesium sulfate, filter off the drying agent, and then remove the solvent from the filtrate *in vacuo* to afford the crude product. Purify the crude product by column chromatography using silica gel and hexane/ethyl acetate to afford the  $\alpha$ -C-glycoside (**7**) in 58%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.27 (d,  $J = 6.5$  Hz,  $\text{CH}(\text{OH})\text{CH}_3$ , 3H), 2.84 (br s,  $\text{CH}(\text{OH})\text{CH}_3$ , 1H), 3.42 (d,  $J = 8.0$  Hz, OH, 1H), 3.57 (dd,  $J = 2.7, 7.4$  Hz, H-1, 1H), 3.63 (m, H-4, 1H), 3.67 (dd,  $J = 5.1, 10.0$  Hz,  $\text{CH}_2\text{OBn}$ , 1H), 3.77 (dd,  $J = 6.2, 10.0$  Hz,  $\text{CH}_2\text{OBn}$ , 1H), 3.79 (t,  $J = 4.8$  Hz, H-3, 1H), 3.99 (ddd,  $J = 2.7, 4.8, 8.0$  Hz, H-2, 1H), 4.07 (m, H-5, 1H), 4.11 (dq,  $J = 6.5, 7.4$  Hz,  $\text{CH}(\text{OH})\text{Me}$ , 1H), 4.64–4.48 (m,  $\text{CH}_2\text{Bn}$ , 6H), 7.35–7.23 (m, ArH, 15H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 19.71, 67.06, 68.05, 68.39, 72.69, 73.09, 73.30, 73.68, 74.25, 74.57, 77.21, 127.62, 127.69, 127.92, 127.99, 128.37, 128.50, 128.56, 137.30, 137.78, 138.07; HRMS (FAB, positive) calculated for  $\text{C}_{29}\text{H}_{35}\text{O}_6$  479.2434, found 479.2422 ( $\text{MH}^+$ ).

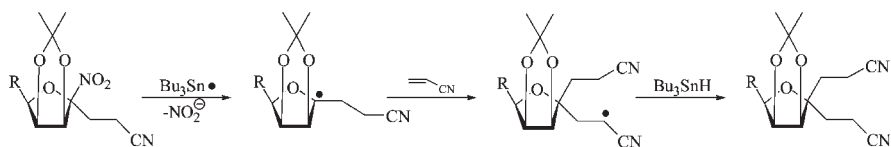
### 11.2.1.3 C-1 nitro carbohydrates

As mentioned above, one inherent drawback with the generation of an anion at the anomeric centre of a carbohydrate is the facile  $\beta$ -elimination of the C-2 substituent. However, anomeric nitro sugars enable the generation of  $\beta$ -glycosides with good selectivity in both the furanoid and pyranoid forms without elimination of the C-2 substituent. Vasella demonstrated that incorporation of a nitro group at C-1 [25] enables, via resonance stabilisation, the generation of stable anomeric anions [26]. Access to the 1-deoxy-1-nitroaldone is achieved via ozonolysis of *N*-glycosyl nitrones [27]. Treatment of the nitrone (**11**) with potassium carbonate and a suitable electrophile then affords the nitro ether (**12**) in 56% yield, via a Henry type reaction. Reductive de-nitration [26] under radical conditions occurs via axial hydrogen transfer to generate the  $\beta$ -C-glycoside (**13**) ( $\beta/\alpha$  85:15) (Scheme 11.3).

The scope of this reaction can be further increased in a number of ways. For example, addition of an electron deficient alkene to the anomeric radical in the denitration process allows the generation of more highly functionalised sugar mimetics (Scheme 11.4) [28].



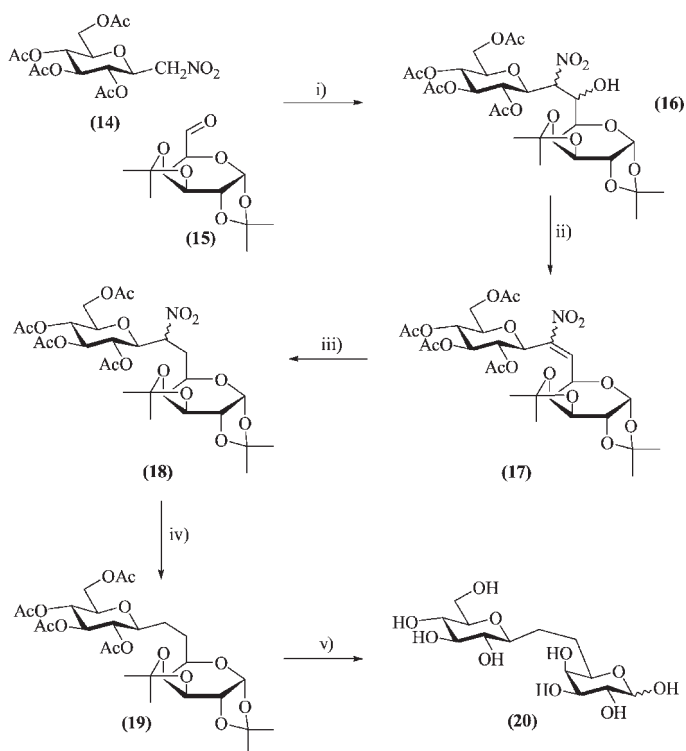
Scheme 11.3



Scheme 11.4

Strong nucleophiles can also be used as an alternative to the radical de-nitration method [29], with the synthesis of disaccharides being facilitated by the use of 1-deoxy-1-nitroaldoses [30].

A fluoride ion mediated condensation of the nitro sugar (**14**) with an *aldehydo* sugar, according to the method of Martin *et al.* [31], provides an expeditious route to a range of  $\beta$ -linked-*C*-disaccharides (**20**). Here removal of the nitro group is achieved under radical conditions (Scheme 11.5).



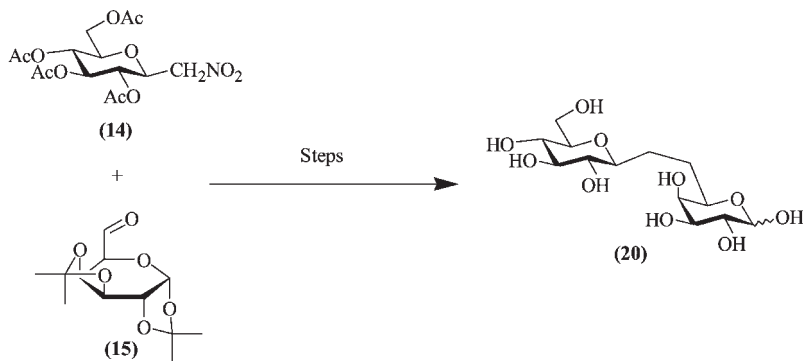
i) KF, CH<sub>3</sub>CN; ii) Ac<sub>2</sub>O, pyr.; iii) NaBH<sub>4</sub>; iv) AIBN, Bu<sub>3</sub>SnH;  
v) a) MeONa, MeOH, b) Amberlite IR-120, H<sub>2</sub>O.

Scheme 11.5

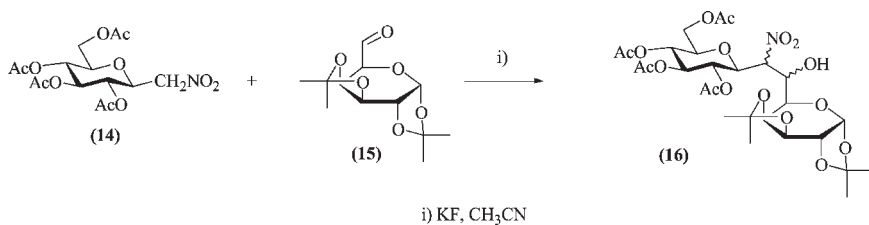


*Method 2*

Synthesis of 8,12-anhydro-6,7-dideoxy-D-glycero-D-gulo-D-galacto-tridecose (**20**) [32].



*Notes and discussion.* This method allows entry to the carba-analogue of 6-*O*- $\beta$ -D-glucopyranosyl-D-galactose in six steps from the glycosylnitromethane (**14**). Since glycosylnitromethanes can be obtained from most free sugars [33], this methodology allows general entry to  $\beta$ -(1,6)-linked C-disaccharides. The reaction has also been applied to open chain aldehydo-hexose derivatives to allow access to the C-analogues of 1,1-linked disaccharides such as  $\beta$ , $\beta$ -trehalose.

*Part 1: condensation of nitro-sugar (14) with aldehyde (15)**Materials.*

3,4,5,6-Tetra-*O*-acetyl-2,6-anhydro-1-deoxy-1-nitro-D-glycero-D-gulo-heptitol (**14**) [34] (4.0 g, 10.2 mmol)

treat as harmful

Aldehyde (**15**) [35] (3.84 g, 14.9 mmol)

treat as harmful

Dry acetonitrile

toxic

Potassium fluoride (0.90 g, 15.6 mmol)	toxic
18-Crown-6 (0.50 g)	harmful, irritant
Water	
Ethyl acetate	irritant
Magnesium sulfate	treat as harmful
Ethyl acetate and hexane for chromatography	toxic, flammable

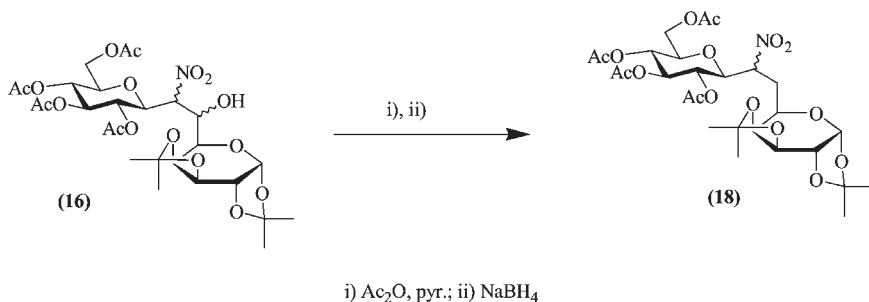
*Equipment.*

Round bottom flask (100 ml) with rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Separatory funnel and conical flasks  
Rotary evaporator  
Equipment for chromatography  
Source of dry nitrogen or argon  
Filter funnel and filter paper

*Procedure.* All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

Once the glassware is dry, equip a 100 ml round bottom flask with a magnetic stirrer bar and rubber septum and flush with dry argon to establish an inert atmosphere. This must be maintained throughout the reaction. Add a solution of the aldehyde (**15**) (3.84 g, 14.9 mmol) in dry CH<sub>3</sub>CN (50 ml). Sequentially add the nitro sugar (**14**) (4.0 g, 10.2 mmol), KF (0.90 g, 15.6 mmol), and 18-crown-6 (0.50 g). Allow the mixture to stir at room temperature for 3 h. Then quench the reaction by the addition of water (200 ml) and ethyl acetate (600 ml). Transfer the solution to a separatory funnel and separate the organic layer and dry with magnesium sulfate, filter off the drying agent, and remove the solvent from the filtrate *in vacuo* to afford the crude reaction product. This should be purified by column chromatography using silica gel and ethyl acetate/hexanes 5:6, as the eluent, to afford the disaccharide (**16**) in 81% yield. IR (Film) 3500 (OH), 1765 (C=O), 1563 and 1378 (NO<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) selected data: 3.68 (1H, dd, *J* = 9.8, 4.9 Hz, H-12), 3.72 (1H, dd, *J* = 1.8, 9.2 Hz, H-5), 4.03 (1H, dd, *J* = 4.9, 12.5 Hz, H-13A), 4.14 (1H, m, H-4), 4.23 (1H, dd, *J* = 4.1, 9.2 Hz, H-6), 4.26 (1H, dd, *J* = 2.4, 12.5 Hz, H-13B), 4.30 (1H, dd, *J* = 4.9, 2.4 Hz, H-2), 4.43 (1H, dd, *J* = 1.8, 8.0 Hz, H-4), 4.62 (1H, *J* = 2.4, 8.0 Hz, H-3), 4.86 (1H, t, *J* = 4.1 Hz, H-7), 5.07 (1H, m, H-7), 5.14–5.16 (2H, m, H-9 and H-10), 5.44 (1H, d, *J* = 4.9 Hz, H-1); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 20.50, 20.53, 20.62, 24.28, 24.81, 25.61, 25.88, 61.33, 67.07, 67.79, 68.82, 69.06, 70.29, 70.38, 70.52, 74.31, 75.14, 76.04, 85.48, 96.19, 109.17, 109.38, 168.87, 169.22, 170.33, 170.87.

*Part 2: de-oxygenation of C-disaccharide (16) to afford C-disaccharide (18)*



*Materials.*

Disaccharide (**16**) (3.01 g, 4.64 mmol)

assume toxic

Dry chloroform (30 ml)

possible  
carcinogen,  
harmful

Acetic anhydride (2.6 ml)

flammable,  
corrosive

Pyridine (1.5 ml)

highly  
flammable,  
harmful

Aqueous hydrochloric acid (20 ml)

horrosive

Saturated NaHCO<sub>3</sub> (2 × 20 ml)

treat as harmful

Water

Magnesium sulfate

irritant

Ethyl acetate and hexane for column chromatography

toxic, flammable

Dichloromethane

harmful

Sodium borohydride (7.6 mg, 0.2 mmol)

flammable, toxic,  
corrosive

Methanol (5 ml)

flammable, toxic

Ethyl acetate (50 ml)

flammable, toxic

*Equipment.*

Round bottom flasks (2 × 50 ml) with rubber septa plus magnetic stirrer bars

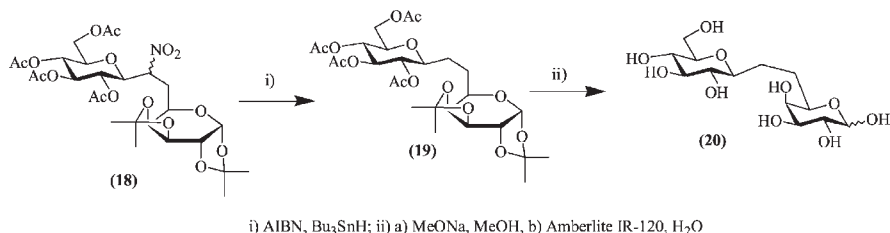
Magnetic stirrer

Source of dry argon or nitrogen

Ice cooling bath  
Dry glass syringes and needles  
Separatory funnel and conical flasks  
Filter funnel and filter paper  
Rotary evaporator  
Equipment for chromatography

All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

Add  $\text{CHCl}_3$  (30 ml) to disaccharide (**16**) (3.01 g, 4.64 mmol) in a 100 ml dry round bottomed flask, under an inert atmosphere, and cool the solution to 0 °C. Add  $\text{Ac}_2\text{O}$  (2.6 ml) and pyridine (1.5 ml) and allow the reaction to warm to room temperature. Leave the reaction stirring at room temperature for 2 h, then quench the reaction by the careful addition of cold aqueous HCl. Transfer the mixture to a separatory funnel and wash the organic phase with saturated  $\text{NaHCO}_3$  (2 × 20 ml) and water (20 ml). Collect the organic phase and dry this with magnesium sulfate, remove the drying agent by filtration, and concentrate the solution *in vacuo*. Purify the crude reaction mixture by flash chromatography using silica gel and ethyl acetate and hexanes 1:1, as the eluent to afford (**17**), as the *Z* isomer, in 91% yield. Transfer (**17**) (256 mg, 0.41 mmol), to a 50 ml dry round bottomed flask and establish an inert atmosphere, which should be maintained throughout the reaction. Add the minimum quantity of  $\text{CH}_2\text{Cl}_2$  necessary to cause dissolution. Cool the flask to 0 °C. Add  $\text{NaBH}_4$  (7.6 mg, 0.2 mmol) in MeOH (5 ml) and leave the reaction to stir until the reaction has gone to completion as evidenced by TLC analysis. Transfer the reaction mixture to a separatory funnel and add ethyl acetate (50 ml) and wash the organic layer with water (2 × 20 ml), dry the organic phase with magnesium sulfate, remove the drying agent by filtration and concentrate the solution *in vacuo*. Purify the crude product thus obtained by flash chromatography using silica gel and ethyl acetate/hexanes 5:8, as the eluent to afford (**18**), as a mixture of stereoisomers at C-7, in 71% yield. Flaky crystals of the major stereoisomer can be obtained by recrystallisation from EtOH. mp 173.2–174.0 °C;  $[\alpha]_{\text{D}}^{22} = -35.4^\circ$  (*c* 0.24,  $\text{CHCl}_3$ ); IR (film) 1760 (C=O), 1530 and 1370;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) selected data: 2.05 (1H, dd, *J* = 2.4, 4.0 Hz, H-6), 2.37 (1H, dd, *J* = 11.6, 4.0 Hz, H-6), 3.66 (1H, m, H-12), 3.69 (1H, dd, *J* = 1.8, 11.0 Hz, H-5), 3.92 (1H, dd, *J* = 4.0, 9.5 Hz, H-8), 4.12 (1H, dd, *J* = 1.8, 8.0 Hz, H-4), 4.13–4.15 (2H, m, H-13A and H-13B), 4.27 (1H, dd, *J* = 2.4, 5.0 Hz, H-2), 4.58 (1H, dd, *J* = 2.4, 8.0 Hz, H-3), 4.94 (1H, dd, *J* = 11.6, 4.0 Hz, H-7), 5.07–5.15 (3H, m, H-9, H-10, H-11), 5.45 (1H, d, *J* = 5.0 Hz, H-1);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 20.53, 20.54, 20.62, 20.65, 24.22, 24.88, 25.70, 25.90, 29.69 (C-6), 61.61 (C-13), 63.59, 67.80, 68.85, 70.45, 70.88, 72.58, 74.36, 76.23, 77.06, 82.90, 96.23 (C-1), 108.82, 109.34, 168.96, 170.37, 170.60.

*Part 3: de-nitration and deprotection of C-disaccharide (18) to afford target (20)**Materials.*

C-Disaccharide ( <b>18</b> ) (384 mg, 0.61 mmol)	assume toxic
AIBN (30 mg)	harmful, explosive
tri-Butyl tin hydride (0.5 ml, 1.83 mmol)	toxic
Sodium methoxide (0.5 M in MeOH, 1 ml)	toxic
Methanol (1 ml)	toxic
Amberlite IR-120 (H <sup>+</sup> ) resin	irritant
Hexane and ethyl acetate for chromatography	irritant
Dichloromethane/hexane for recrystallization	harmful

*Equipment.*

Round bottomed flask (50 ml) with rubber septum and magnetic stirrer bar  
 Two necked flask (10 ml) with reflux condensor, rubber septum and magnetic stirrer bar  
 Source of dry argon or nitrogen  
 Dry glass syringes and needles  
 Glass sinter funnel  
 Separatory funnel and conical flasks  
 Stirrer hotplate  
 Rotary evaporator  
 Equipment for chromatography

All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

Transfer (**18**) (384 mg, 0.61 mmol) to a dry 5 ml two necked flask equipped with a reflux condensor and establish an inert atmosphere, that should be maintained throughout the reaction. Add  $\text{Bu}_3\text{SnH}$  (0.5 ml, 1.83 mmol) and AIBN (30 mg). Heat the mixture under reflux for no more than 30 min. Remove the solvent *in vacuo* and purify the residue by flash chromatography using silica gel and ethyl acetate/hexanes 5:8, as the eluent to afford (**19**) in 57% yield. Transfer (**19**) to a dry 50 ml round bottomed flask and establish an inert atmosphere that should be maintained throughout the reaction. Dissolve (**19**) in MeOH (15 ml). Add a 0.5 M solution of MeONa in MeOH (1 ml) and leave the solution to stir at room temperature for 30 min. Then neutralize the reaction mixture with methanol-washed Amberlite IR-120 ( $\text{H}^+$ ) resin. Remove the resin by filtration to afford the organic filtrate which is concentrated *in vacuo*. Re-dissolve the residue in water (10 ml) and add Amberlite IR-120 ( $\text{H}^+$ ) ion-exchange resin, previously washed with hot (70 °C) water, to the solution and heat the mixture at 70 °C for 2.5 h. Remove the resin from the cooled solution by filtration, and concentrate the filtrate to give pure (**20**) in 89% yield.

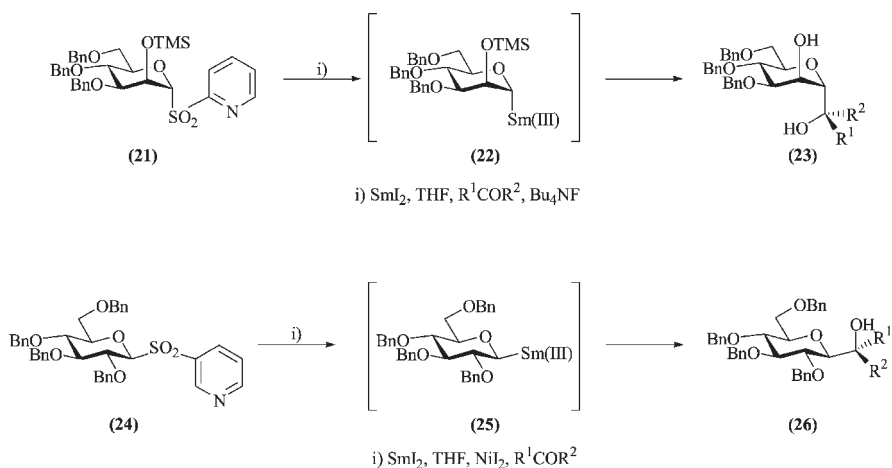
Data for (**19**) [32]  $[\alpha]_{\text{D}}^{22} = -45.6^\circ$  (*c* 1.8,  $\text{CHCl}_3$ ); IR (Film) 1760 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.32, 1.35, 1.46, 1.50 ( $4 \times \text{s}$ ,  $\text{CMe}_2$ , 12H), 1.20–1.85 (m, H-6, H-7, 4H), 2.00, 2.02, 2.03, 2.09 ( $4 \times \text{s}$ , OAc, 12H), 3.43 (ddd,  $J = 1.9, 9.8, 9.8$  Hz, H-8, 1H), 3.60 (ddd,  $J = 2.3, 4.7, 9.8$  Hz, H-12, 1H), 3.67 (ddd,  $J = 1.8, 2, 10$  Hz, H-5, 1H), 4.07 (dd,  $J = 12.3, 2.3$  Hz, H-13, 1H), 4.11 (dd,  $J = 1.8, 7.9$  Hz, H-4, 1H), 4.27 (dd,  $J = 4.7, 12.3$  Hz, H-13, 1H), 4.28 (dd,  $J = 2.3, 5.0$  Hz, H-2, 1H), 4.58 (dd,  $J = 2.3, 7.9$  Hz, H-3, 1H), 4.88 (dd,  $J = 9.5, 9.8$  Hz, H-9, 1H), 5.06 (dd,  $J = 9.5, 9.8$  Hz, H-11, 1H), 5.15 (dd,  $J = 9.5, 9.5$  Hz, H-10, 1H), 5.51 (d,  $J = 5.0$  Hz, H-1, 1H);  $^{13}\text{C}$  NMR 20.60, 20.65, 20.75, 20.76 ( $4 \times \text{OCOMe}$ ), 24.27, 24.92, 25.96, 26.05, 26.20, 28.08 ( $2 \times \text{CMe}_2$ , C-6,7), 62.28 (C-13), 67.65, 68.63, 70.43, 70.86, 71.93, 73.00, 74.58, 75.65, 77.94 (C-2-5, C-8-12), 96.43, (C-1), 108.32, 108.99 ( $2 \times \text{CMe}_2$ ), 169.50, 169.69, 170.45, 170.73 ( $4 \times \text{OCOMe}$ ). Anal. Calcd for  $\text{C}_{27}\text{H}_{40}\text{O}_{14}$  C, 55.10; H, 6.85. Found: C, 54.92; H, 6.85.

### 11.2.2 Reductive samarium method

The chemistry of anomeric samarium species bears similarities to that of the organolithium species discussed earlier. However, in contrast, they are highly stable and do not undergo  $\beta$ -elimination. The introduction of  $\text{SmI}_2$  as a reducing agent can be credited to Kagan [36] and has since been utilised in numerous synthetic procedures [37]. Sinaÿ and Beau [38] introduced  $\text{SmI}_2$  chemistry into C-glycoside synthesis via intermolecular reactions with appropriate electrophiles, and intramolecular reactions of silyl tethered substrates. Treatment of the aryl sulfones (**21/24**) with  $\text{SmI}_2$  affords the nucleophilic intermediates (**22/25**) via two single electron transfer processes. Introduction of a suitable electrophile

e.g. cyclohexanone, affords the  $\alpha$ -C-mannopyrannoside (**23**) in 86% yield and with excellent stereoselectivity ( $\alpha/\beta$  86/1). Identical reactions in the glucose and galactose series disappointingly led to the generation of  $\beta$ -C-glycosides in moderate yields [39]. However, more recent work by Beau *et al.* [40]. led to the inclusion of nickel(II) iodide as a metal catalyst. Consequently subsequent reactions (in the *gluco* series) have seen a significant increase in yields. For example, when cyclohexanone is used as the electrophile [40] exclusive entry to the  $\beta$ -C-glucopyrannoside (**26**) is achieved in 94% yield (Scheme 11.6).

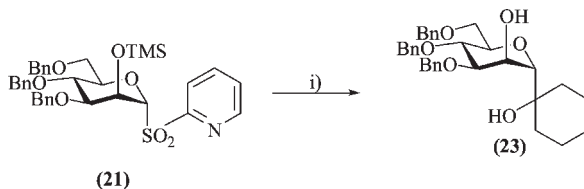
The interesting stereochemical outcome of the reactions can be explained by the preference of the  $\text{Sm}^{3+}$ -substituent to occupy the more stable equatorial position within the transition state. Conveniently, the progress of the reaction can be monitored visually by the colour change brought about by the generation of the samarium (III) salts which can be seen as light yellow or orange [37c]. Alternative anomeric substituents have been employed to facilitate the initial single electron transfer process [37a, 41], and the method has been employed towards the synthesis of disaccharide mimics [42]. A further evolution of this intermolecular approach is the inclusion of an *N*-acetyl group at C-2. The incorporation of such a group was expected to be problematic due to the acidity of the proton on the acetamido group. However, treatment under Barbier conditions produced a mixture of the C-glycosides in good yield. Galactosamine [43] and glucosamine [44] substrates preferentially afforded  $\alpha$ -linked C-glycosides, a result which is in contrast with the initial findings. It was hypothesised that complexation between the metal and the acetamido group at C-2, brought about by a conformational change to allow the thermodynamically more stable intermediate to be formed, promoted this interaction and lead to the generation of the  $\alpha$ -anomer [43].



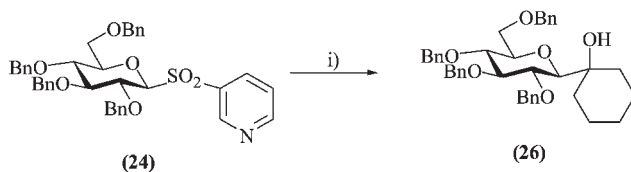
**Scheme 11.6**

*Method 3*

Stereoselective synthesis of 1,2-*trans*-C-glycosides via glycosyl samarium (III) compounds [40].



i) SmI<sub>2</sub>, THF, cyclohexanone, Bu<sub>4</sub>NF



i) SmI<sub>2</sub>, THF, NiI<sub>2</sub>, cyclohexanone

*Notes and discussion.* This method describes the synthesis of α-C-mannosides and β-C-glucosides via the direct coupling of pyridyl sulfones with cyclohexanone in the presence of samarium diiodide. These reactions must be performed under Barbier conditions since addition of the carbonyl compound even 20 s after the addition of SmI<sub>2</sub> does not lead to any condensation product. Under the optimum conditions, the bimolecular organosamarium-carbonyl condensation for mannopyranosides is favoured over the unimolecular elimination process, even though a favourable *trans*-diaxial relationship exists between the C-1-Sm and C-2-OSiMe<sub>3</sub> units. When β-glucosyl sulfones are utilised in the samarium mediated reaction, the β-C-glucosides are formed in only moderate yields due to competing elimination processes. Acceptable yields can, however, be achieved by replacing the TMS group with the bulky TBDMS group. Alternatively, a catalytic quantity of nickel(II) iodide (1 mol%) can be added to the reducing agent. The reaction has also been extended to include carbohydrate derived ketones, allowing entry to C-linked disaccharides.

*Materials.*

Sulfone (21) (85 mg, 0.13 mmol) [45] or sulfone (24) (25 mg, 37.5 μmol) [45]      treat as harmful

Samarium iodide (0.1 M in THF, 2.7 ml, 0.27 mmol)      irritant

Cyclohexanone (20 μl, 0.19 mmol)      irritant



Saturated aqueous ammonium chloride	irritant
Dichloromethane	harmful
Sodium sulfate	irritant
tetra-Butyl ammonium fluoride (1.0 M in THF)	flammable, toxic, corrosive
Dry THF (5 ml)	flammable, toxic
NiI <sub>2</sub> in THF (0.01 M, 10 ml)	toxic, irritant
Heptane, cyclohexane and ethyl acetate for chromatography	irritant, flammable

### Equipment.

Round-bottomed flasks (2 × 10 ml) with rubber septa and magnetic stirrer bars  
 Magnetic stirrer  
 Source of dry nitrogen or argon  
 Ice cooling bath  
 Dry glass syringes and needles  
 Separatory funnel and conical flasks  
 Filter funnel and filter paper  
 Rotary evaporator  
 Equipment for chromatography

**Procedure.** All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

For entry to the  $\alpha$ -C-mannoside (**23**): Once the glassware is dry equip a 10 ml round bottom flask with a magnetic stirrer bar and rubber septum and establish an inert atmosphere. This should be maintained throughout the reaction. Then add sulfone (**21**) (85 mg, 0.13 mmol) and cyclohexanone (20  $\mu$ l, 0.19 mmol) to the flask. Add a 0.1 M solution of SmI<sub>2</sub> in THF (2.7 ml, 0.21 mmol) to the solution, at 20 °C. After stirring for 10 min quench the reaction by the addition of aq. saturated ammonium chloride and transfer the mixture to a separatory funnel. Extract the mixture twice with DCM. Combine the organic fractions and wash twice with water, dry with sodium sulfate, remove the drying agent by filtration, and remove the solvent *in vacuo* to afford the crude product. Redissolve this residue in dry THF (5 ml) and cool to 0 °C (under an inert atmosphere, that should be maintained during the reaction). Add Bu<sub>4</sub>NF in THF (135  $\mu$ l, 0.135 mmol) and leave the reaction to stir at 0 °C for 5 min. Quench the reaction by the addition of water and DCM, and transfer the mixture to a separatory funnel. Wash the organic phase twice with water, dry with sodium sulfate, remove the drying agent by filtration and remove the solvent *in vacuo* to afford the crude deprotected C-glycoside. The residue can then be purified by column chromatography using silica gel and heptane/ethyl acetate 7:1  $\rightarrow$  3:1 to afford the  $\alpha$ -anomer (**23**) in 86% yield. (R = cyclohexanone)

$[\alpha]_D^{20} = 8.0$  ( $c$  0.8,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.47–1.71 (m,  $\text{CH}_2$ , 10H), 2.50 (s, OH, 1H), 2.73 (d,  $J = 5.2$  Hz, OH-2, 1H), 3.48 (d,  $J = 7.7$  Hz, H-1, 1H), 3.62 (dd,  $J = 10.2, 5.0$  Hz, H-6, 1H), 3.71 (dd,  $J = 10.2, 5.9$  Hz, H-6', 1H), 3.72 (dd,  $J = 5.7, 4.1$  Hz, H-4, 1H), 3.87 (dd,  $J = 5.7, 3.9$  Hz, H-3, 1H), 4.11 (ddd,  $J = 5.9, 5.0, 4.1$  Hz, H-5, 1H), 4.16 (ddd,  $J = 7.7, 5.2, 3.9$  Hz, H-2, 1H), 4.49 (s,  $\text{CH}_2\text{Ph}$ , 2H), 4.51, 4.56, 4.60, 4.61 (4d,  $J = 12$  Hz,  $\text{CHPh}$ , 4H), 7.32–7.38 (m, 3Ph, 15H).

For entry to the  $\beta$ -C-glucoside (**26**): add a solution of  $\text{NiI}_2$  in dry THF (0.01 M, 10 ml) to a solution of  $\text{SmI}_2$  in THF (0.1 M, 10 ml) under argon. Cool this solution (1.03 ml, 94  $\mu\text{mol}$  of  $\text{SmI}_2$ ) to  $0^\circ\text{C}$  and add to a solution of pyridyl sulfone (**24**) (25 mg, 37.5  $\mu\text{mol}$ ) and cyclohexanone (12  $\mu\text{l}$ , 116  $\mu\text{mol}$ ) in THF (0.5 ml) at  $0^\circ\text{C}$  under argon. After stirring at  $0^\circ\text{C}$  for 30 min, add a saturated aqueous ammonium chloride solution. Transfer the reaction mixture to a separatory funnel and extract the reaction mixture twice with DCM, and wash the organic fractions twice with water. Dry the organic phases with sodium sulfate, remove the drying agent by filtration, and concentrate the solution *in vacuo*. Purification by flash chromatography using silica gel and cyclohexane/ethyl acetate 6:1, as the eluent affords the  $\beta$ -glucoside (**26**) in 94% yield. ( $\text{R} = \text{cyclohexanone}$ )  $[\alpha]_D^{20} = +3.0$  ( $c$  2.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.73–1.45 (m,  $5 \times \text{CH}_2$ , 10H), 3.15 (d,  $J = 9.2$  Hz, H-1, 1H), 3.30 (br s, OH, 1H), 3.44 (ddd,  $J = 3.2, 9.7$  Hz, H-5, 1H), 3.64 (dd,  $J = 8.8, 9.7$  Hz, H-4, 1H), 3.69–3.70 (m, H-6, 2H), 3.74, 3.81 ( $2 \times \text{t}$ ,  $J \approx 9$  Hz, H-2, H-3, 2H), 4.57, 4.62, 4.63, 4.73, 4.82, 4.86, 4.98, 5.05 ( $8 \times \text{d}$ ,  $J = 11$  Hz,  $\text{CH}_2\text{Ph}$ , 8H), 7.19–7.37 (m, ArH, 20H); HRMS (ES)  $\text{C}_{40}\text{H}_{46}\text{NaO}_6$   $[\text{M}^+\text{Na}]$ , calculated 645.3192, found 645.3198.

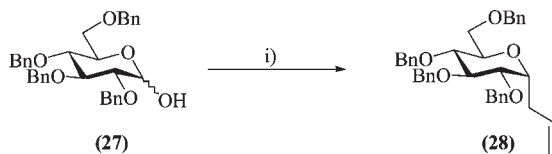
### 11.2.3 Use of the anomeric centre as an electrophilic species

The most common and frequently employed method for *O*-glycosylation exploits the anomeric centre's electrophilic character. In traditional *O*-glycosylations, this electrophilic character is enhanced by treatment of the donor with a Lewis acid, which promotes the generation of an oxonium ion. In such instances the stereochemical outcome can be controlled by external factors such as solvent, and more directly by neighbouring group participation. A certain degree of stereochemical control can be introduced in the synthesis of *C*-glycosides via the aforementioned participating groups [46]; however, such groups are not always present within the substrates. The following examples therefore demonstrate the generation of  $\alpha/\beta$  glycosides via alternative means.

#### *Lactols*

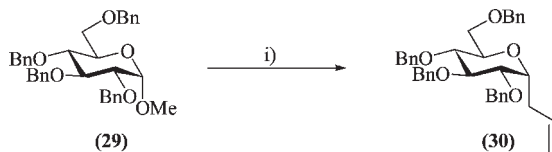
In 1982 Kishi [47] successfully synthesised the  $\alpha$ -C-glycoside (**28**) from 2,3:4,6-tetra-*O*-benzylglucose (Scheme 11.7).

Employing traditional glycosylation techniques, and exploiting the nature of carbon based nucleophiles to attack from the  $\alpha$ -face, the *C*-glycoside (**28**) was prepared in 50% yield with good 10:1  $\alpha/\beta$  stereoselectivity. The incorporation of



i) allyltrimethylsilane,  $\text{BF}_3 \cdot \text{OEt}_2$ , MeCN

**Scheme 11.7**



i) allyltrimethylsilane, TMSOTf, MeCN

**Scheme 11.8**

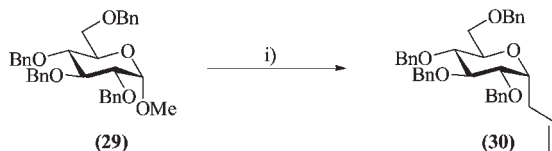
an activating group at the C-1 position i.e. *p*-nitrobenzoyl, saw an increase in yield to 80% whilst maintaining the good selectivity shown under the original conditions.

In 1984 Hosomi *et al.* [48] further developed this work by treating methyl 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranoside (**29**) with TMSOTf at room temperature for 16 h. This afforded the  $\alpha$ -isomer (**30**) in 86% yield and 10:1  $\alpha/\beta$  selectivity without the need for incorporation of activating groups at the anomeric position (Scheme 11.8).

Evolution of this method has seen the use of a range of alkenes, alkynes and enol ethers as nucleophiles, all of which yield the C-glycosides in high yield and with good selectivity. Subsequently Kishi [49] and Nicotra [50] have developed this methodology for the synthesis of natural product mimics. In addition, recent work by Bennek and Gray [51] has revealed the stereospecific introduction of allyl groups to the C-1 position using fully deprotected sugar substrates. This work provides a valuable alternative to the method of Kishi [47, 49, 52] and Hosomi [48] as removal of the protecting groups under traditional hydrogenation conditions may result in the reduction of the allyl substituent, or other functionalities susceptible to these conditions within the molecule.

#### Method 4

Highly stereoselective C-allylation of glycopyranosides with allylsilanes, catalysed by silyl triflate or iodosilane [48].



i) allyltrimethylsilane, TMSOTf, MeCN

*Notes and discussion.* Methyl  $\alpha$ -D-glucopyranosides and mannopyranosides and  $\alpha$ -D-glycopyranosyl chlorides can react with allylsilanes via activation with trimethylsilyl trifluoromethanesulfonate or iodotrimethylsilane to stereoselectively afford the corresponding  $\alpha$ -C-allylated glycopyranosides in excellent yields. The yields of the reactions depend on the solvent and the amount of catalyst employed. Acetonitrile is the most suitable solvent whereas dichloromethane, the most commonly used solvent for allylation reactions using allylsilanes, does not afford satisfactory results. The reactions proceed very slowly when promoted by less than 5 mol% of the catalyst, however, the use of 50 mol% of catalyst is sufficient to force the reactions to completion.

*Materials.*

2,3,4,6-Tetra- <i>O</i> -benzyl methyl $\alpha$ -D-glucopyranoside (1 equiv.)	treat as harmful
Allyltrimethylsilane (2 equiv.)	flammable, irritant
Acetonitrile (2 ml/equiv.)	toxic
Trimethylsilyl trifluoromethanesulfonate (0.2 equiv.)	flammable, moisture sensitive
Pyridine	highly flammable, harmful
Aq. sodium hydrogen carbonate	treat as harmful
Magnesium sulfate	treat as harmful

*Equipment.*

Round-bottomed flask with rubber septum plus magnetic stirrer bar  
Magnetic stirrer  
Source of dry argon or nitrogen  
Ice cooling bath  
Dry glass syringes  
Separatory funnel and conical flasks  
Filter funnel and filter paper  
Rotary evaporator  
Equipment for chromatography  
HPLC equipment

*Procedure.* All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

Once the glassware is dry, equip a round bottom flask with a magnetic stirrer bar and rubber septum and establish an inert atmosphere. This should be maintained throughout the reaction. Add 2,3,4,6-tetra-*O*-benzyl methyl- $\alpha$ -D-glucopyranoside (**29**) (1 equiv.), allylsilane (2 equiv.) and acetonitrile (2 ml/equiv.) and cool the reaction mixture to 0 °C. Add trimethylsilyl trifluoromethanesulfonate (0.2 equiv.) and pyridine to the reaction mixture and leave the reaction to stir at room temperature for 16 h. Quench the reaction by the careful addition of saturated sodium hydrogencarbonate solution and transfer the mixture to a separatory funnel. Extract the mixture with ethyl acetate, dry the organic layers with magnesium sulfate, remove the drying agent by filtration, and concentrate the solution *in vacuo*. Purification by chromatography followed by separation of the anomers by HPLC affords the  $\alpha$ -isomer (**30**) in 86% yield. Data [69] [ $\alpha$ ]<sub>D</sub> = +36.5 (*c* 2.19, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.51 (m, 2H), 3.61–3.68 (m, 3H), 3.72 (dd, *J* = 3.3, 10.5 Hz, 1H), 3.77 (dd, *J* = 5.5, 9.4 Hz, 1H), 3.82 (dd, *J* = 7.5, 9.4 Hz, 1H), 4.16 (ddd, *J* = 5.0, 5.1, 10.5 Hz, 1H), 5.09 (d, *J* = 10.2 Hz, 1H), 5.13 (dd, *J* = 1.3, 17.2 Hz, 1H), 5.83 (dddd, *J* = 6.7, 7.1, 10.2, 17.2 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 29.81, 69.01, 71.18, 73.06, 73.46, 73.71, 75.03, 75.38, 78.15, 80.08, 82.41, 116.181, 127.53, 127.56, 127.67, 127.74, 127.79, 127.83, 127.87, 127.93, 128.29, 128.35, 128.39, 134.75, 138.13, 138.25, 138.78; MS (FAB, NaI) *m/z* 587 (M<sup>+</sup>Na).

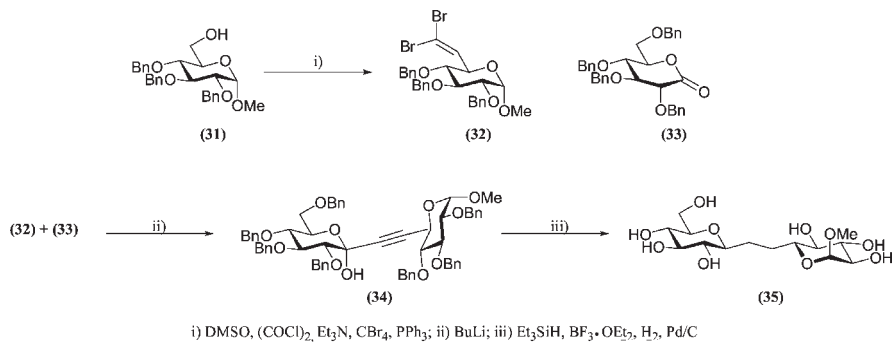
### Lactones

One pioneering experiment that demonstrates the utility of sugar lactones for C-glycoside synthesis was carried out by Sinaÿ in the synthesis of the first C-linked disaccharide in 1983 [53]. Since this early work the inherent ability of sugar lactones to afford  $\beta$ -C-linked glycosides has been utilised in numerous syntheses [54]. Sinaÿ's approach saw the treatment of lactone (**33**) with the acetylenic anion, derived from the vinyl dibromide (**32**). This afforded the hemiacetal (**34**) in 92% yield (Scheme 11.9). Reduction via Et<sub>3</sub>SiH and BF<sub>3</sub>·OEt<sub>2</sub> yielded exclusive entry to the  $\beta$ -linked C-disaccharide (**35**) after hydrogenation with H<sub>2</sub> Pd/C in 52% yield.

Subsequent work by Sinaÿ *et al.* has seen the optimisation of the final reduction step allowing access to either the *E*- or the *Z*- isomer [55] thus further developing the scope of this reaction. Kraus and Molina [56] and Czernecki and Ville [57] have further exploited this reaction. Treatment of lactone (**33**) with a range of organometallic nucleophiles (Table 11.2) exclusively gave the corresponding  $\beta$ -C-glycosides (**36**) in excellent yields (Scheme 11.10).

The drawback with this, and all other syntheses employing this methodology, is the need for protecting groups that are stable to the reduction conditions employed in the final step.

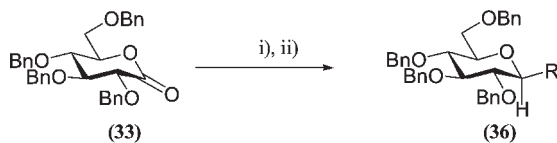
This approach was also explored by Dondoni who in 1993 [58] employed thiazole, a masked formyl group, as the nucleophile in the reaction with sugar lactones. One advantage of the thiazole group is its flexibility; the group can be easily introduced, is stable to a wide range of reaction conditions and can easily be removed under mild conditions [59]. Subsequent removal of the protecting group



Scheme 11.9

Table 11.2

Entry	RM	Yield (%)
1		88
2		65
3	CH <sub>2</sub> =CHMgBr	60
4		95
5		80
6		78

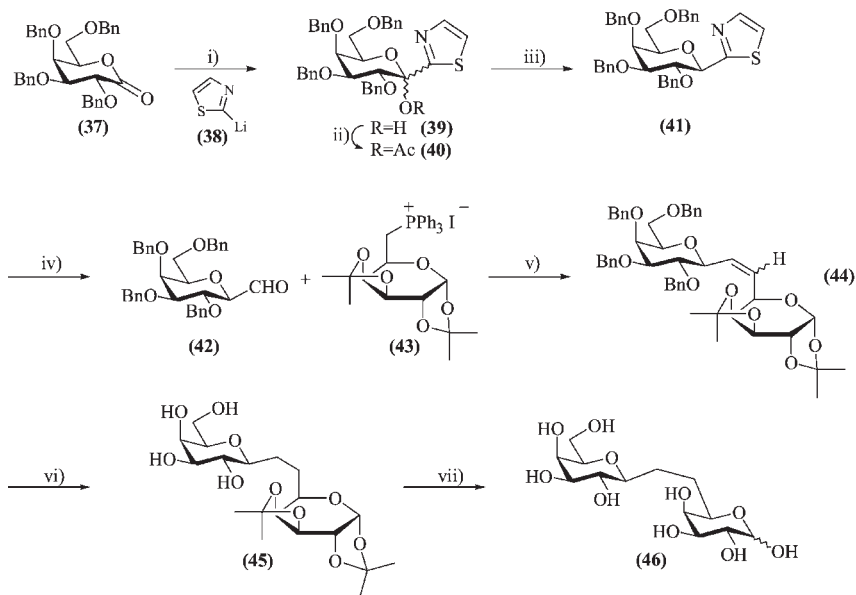


i) RM, THF; ii)  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{Et}_3\text{SiH}$

**Scheme 11.10**

exposes the aldehyde functionality which Dondoni employed successfully in the synthesis of *C*-disaccharides via Wittig olefination [60]. Thus 2-lithiothiazole (**38**) was reacted with tetra-*O*-benzylgalactonolactone (**37**) to afford the *C*-glycoside (**39**) in 78% yield (Scheme 11.11).

Acetylation followed in essentially quantitative yield to afford (**40**). In this case the  $\alpha$ -linked product is formed, but altering the reaction conditions [61] can reverse this. Reduction proceeds via Lewis acid catalysed removal of the acetate group to afford the charged oxonium species. Hydride delivery from the least hindered face ( $\alpha$ -) then occurs to afford the  $\beta$ -*C*-glycoside (**41**) in 96% yield. Conversion of the thiazole ring to the aldehyde group was facilitated by *N*-methylation, reduction with sodium borohydride and hydrolysis to produce (**42**) in 80% yield [61].



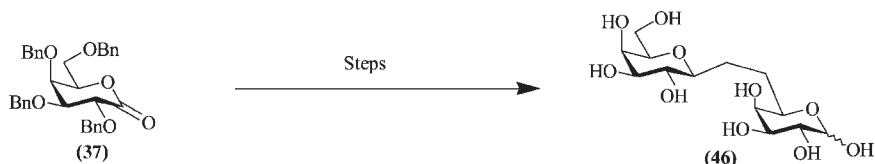
i) THF,  $-78^\circ\text{C}$ , (**38**), 1h; ii)  $\text{Et}_3\text{N}$ ,  $\text{Ac}_2\text{O}$ , rt; iii)  $\text{CH}_2\text{Cl}_2$ ,  $\text{Et}_3\text{SiH}$ , TMSOTf, rt; iv) (a)  $\text{CH}_3\text{CN}$ , MeOTf, (b) MeOH,  $\text{NaBH}_4$ , (c)  $\text{CH}_3\text{CN}$ - $\text{H}_2\text{O}$ ,  $\text{HgCl}_2$ ; v) BuLi,  $-30^\circ\text{C}$ , THF-HMPA; vi)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2$ ; vii) Amberlite IR-120,  $\text{H}_2\text{O}$ .

**Scheme 11.11**

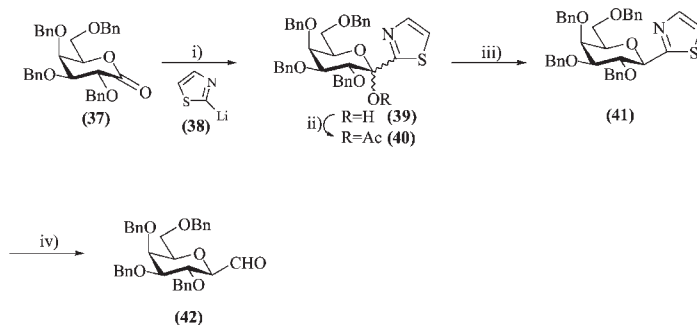
Wittig olefination ensues affording (**44**) in 76% yield, with reduction and deprotection affording the fully deprotected *C*-disaccharide (**46**) in 77%.

### Method 5

Multi-step synthesis of *C*-linked disaccharides using thiazolyketoses [58–61].



*Part 1: addition of 2-lithiothiazole to 2,3,4,6-tetra-O-benzyl-D-galactonolactone (37) and manipulation to afford aldehyde (42)*



i) THF,  $-78^{\circ}\text{C}$ , (**38**), 1h; ii)  $\text{Et}_3\text{N}$ ,  $\text{Ac}_2\text{O}$ , rt; iii)  $\text{CH}_2\text{Cl}_2$ ,  $\text{Et}_3\text{SiH}$ , TMSOTf, rt; iv) (a)  $\text{CH}_3\text{CN}$ , MeOTf, (b) MeOH,  $\text{NaBH}_4$ , (c)  $\text{CH}_3\text{CN-H}_2\text{O}$ ,  $\text{HgCl}_2$

*Notes and discussion.* The first and key step of this procedure involves the stereoselective addition of 2-lithiothiazole to the sugar lactone to afford a ketol. The thiazole ring is used as a masked formyl group, however other formyl anion equivalents could also be utilised to generate the glycosyl aldehyde from the sugar lactone [62]. When alternative glycosyl donors such as acetate or trichloroacetimidate donors were used in the reaction with 2-lithiothiazole, under Lewis acidic conditions, formation of the target ketol product did not occur. As noted in the procedure below, addition of 2-lithiothiazole, generated *in situ* and at low temperature from 2-bromothiazole and *n*-butyl lithium, should generate a homogeneous and slightly yellow solution. Formation of a brown/red solution or a precipitate indicates that decomposition products have formed. The organometallic species can also be formed in THF, but in these cases a milky white suspension forms due to the poorer solubility of the reagent in THF. It is recommended in the source reference that the 2-lithiothiazole is generated from freshly distilled 2-bromothiazole and a good source of *n*-butyl lithium in well purified and strictly anhydrous solvent. It is also noted that the reaction afforded



better yields when carried out with 5–10 mmol of the sugar lactone. The hemiacetal formed in the synthesis can be converted into the kinetic or thermodynamic acetylated derivative by acetylation of its hydroxy group either *in situ* or after isolation of the product. Thus entry to either epimeric acetate is possible, allowing eventual stereoselective entry to either epimer of the C-linked disaccharide, by simply varying the conditions utilised to effect acetylation. Silane reduction is then utilised to effect removal of the acetoxy group. The substrate/silane ratio was found to greatly influence the efficiency of the reaction with a larger excess of the Lewis acid increasing the formation of the anhydro derivative byproducts. The use of acetonitrile as solvent was also detrimental with 1-acetamido derivatives being formed via anomeric acetonitrilium ions. Finally, the silane reduction was found not to proceed at low temperature ( $-20-0^{\circ}\text{C}$ ). The final step of the methodology, conversion of the thiazole ring to the formyl group, is effected under an essentially neutral and mild one-pot method. Copper(II) chloride was investigated for effecting metal mediated hydrolysis, but better yields were generally obtained using mercury(II) chloride, and it is the latter reagent that is utilised in the procedure below.

#### Materials.

2,3,4,6-Tetra- <i>O</i> -benzyl-D-galactonolactone ( <b>37</b> ) [61] (5.00 g, 9.29 mmol)	treat as harmful
<i>n</i> -BuLi (1.6 M solution in hexane, 7.6 ml)	flammable, corrosive, moisture sensitive
Dry ether	irritant, flammable
2-Bromothiazole (1.80 g, 11.10 mmol)	irritant
Dry tetrahydrofuran	flammable, irritant
Phosphate buffer (pH = 7) (200 ml)	irritant
Dichloromethane	harmful
Magnesium sulfate	irritant
Ethyl acetate	highly flammable
Triethylamine (2 ml)	highly flammable, corrosive
Acetic anhydride (2 ml)	flammable, corrosive
4 Å powdered molecular sieves (1.5 + 3.3 g)	treat as harmful

Triethylsilane (3.6 ml, 22.5 mmol)	highly flammable, irritant
Trimethylsilyl trifluoromethanesulfonate (1163 $\mu$ l, 6.3 mmol)	harmful, irritant
Celite <sup>®</sup>	harmful, irritant
Cyclohexane	highly flammable
Methyl triflate	harmful, irritant
Anhydrous acetonitrile (15.3 ml)	toxic, flammable
Methanol	highly flammable
Sodium borohydride (142 mg, 3.7 mmol)	flammable, toxic
Water	
Acetone	highly flammable
Mercury chloride (447 mg, 1.64 mmol)	highly toxic
20% Aqueous KI	sensitising, hygroscopic
Florisil (100–200 mesh)	treat as harmful

*Equipment.*

Round bottomed flasks with rubber septa plus magnetic stirrer bars  
 Magnetic stirrer  
 Source of dry nitrogen or argon  
   – 78, – 30 and 0 °C cooling baths  
 Dry glass syringes with needles  
 Distillation kit (to freshly distil 2-bromothiazole)  
 Separatory funnel and conical flasks  
 Filter funnel and filter paper  
 Sinter funnel  
 Water bath  
 Rotary evaporator  
 Equipment for chromatography

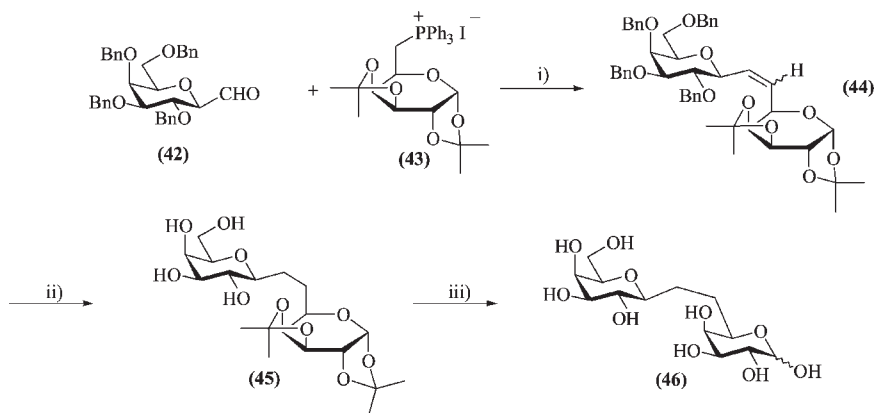
*Procedure.* All glassware must be dried in an oven prior to use, since each stage of the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

Once the glassware is dry, equip a round bottom flask with a magnetic stirrer bar and rubber septum. Establish an inert atmosphere, and this should be maintained throughout the reaction. Add anhydrous ether (17 ml) to the flask, cool to – 78 °C by means of external cooling and add *n*-BuLi (7.6 ml, 12.10 mmol of a 1.6 M solution in hexane) via syringe. Then add freshly distilled 2-bromothiazole

(1.80 g, 11.10 mmol) in dry ether (4.3 ml), dropwise, over a period of 30 min. Stir the yellow solution for 20 min, then slowly add a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactonolactone (**37**) (5.00 g, 9.29 mmol) in anhydrous THF (17 ml) over a period of 25 min. Stir the mixture for a further 20 min at  $-78^{\circ}\text{C}$  then allow the solution to warm to  $-65^{\circ}\text{C}$ . Pour the solution into 200 ml of phosphate buffer (pH = 7) and transfer to a separatory funnel. Extract the aqueous layer with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 100$  ml). Dry the organic fractions with magnesium sulfate, filter to remove the drying agent, and then remove the solvent *in vacuo* to afford the crude product. Purification can be achieved by flash chromatography using silica gel and petroleum ether/ethyl acetate 5:2, as the eluent. The compound is isolated as a white crystalline solid in 78% yield. Transfer the product, 2,3,4,6-tetra-*O*-benzyl-1-(2-thiazolyl)- $\alpha$ -D-galactopyranose (**39**) (1.50 g, 2.40 mmol) to a dry round bottomed flask and establish an inert atmosphere. This should be maintained throughout the reaction. Add  $\text{CH}_2\text{Cl}_2$  (6 ml), triethylamine (2 ml) and acetic anhydride (2 ml). Leave the reaction to stir at room temperature overnight. Concentrate the solution *in vacuo* to afford (**40**) in 100% yield. Add anhydrous  $\text{CH}_2\text{Cl}_2$  (18 ml) to 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-1-(2-thiazolyl)- $\alpha$ -D-galactopyranose (**40**) (1.50 g, 2.25 mmol) under an inert atmosphere. Add activated 4 Å powdered molecular sieves (1.5 g) and stir the suspension at room temperature. Then add  $\text{Et}_3\text{SiH}$  (3.6 ml, 22.5 mmol) and TMSOTf (1163  $\mu\text{l}$ , 6.3 mmol) and stir the mixture at room temperature for 50 min. After this time, neutralize the reaction by addition of triethylamine, dilute the mixture with  $\text{CH}_2\text{Cl}_2$ , filter the suspension through Celite<sup>®</sup>, and concentrate the filtrate *in vacuo*. Purification can be achieved by flash chromatography using silica gel and cyclohexane/ethyl acetate 5:2, as the eluent. Compound **41** is isolated as a residue in 96% yield. Add 2-(2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-galactopyranosyl)thiazole (**41**) (1.00 g, 1.64 mmol) and activated 4 Å powdered molecular sieves (3.3 g) to a round bottomed flask and establish an inert atmosphere, that should be maintained throughout the reaction. Add anhydrous  $\text{CH}_3\text{CN}$  (15.3 ml). Stir the suspension at room temperature for 10 min, then add methyl triflate (240  $\mu\text{l}$ , 2.1 mmol) and stir the suspension at room temperature for 15 min. Filter the suspension through Celite<sup>®</sup> and concentrate the filtrate to dryness. Resuspend the *N*-methylthiazolium salt in dry MeOH (15.3 ml) in a dry round bottomed flask and establish an inert atmosphere, that should be maintained throughout the reaction. Cool the mixture to  $0^{\circ}\text{C}$  by means of external cooling. Treat the suspension with  $\text{NaBH}_4$  (142 mg, 3.7 mmol) and stir the mixture at room temperature for an additional 10 min. Dilute the suspension with acetone (22 ml), filter the mixture through Celite<sup>®</sup>, and concentrate the filtrate *in vacuo*. Add  $\text{HgCl}_2$  (447 mg, 1.64 mmol) to a solution of the crude thiazolidine in 10:1  $\text{CH}_3\text{CN}-\text{H}_2\text{O}$  (15.3 ml). Stir the mixture for 15 min and then filter the suspension through Celite<sup>®</sup>. Remove the acetonitrile from the filtrate via evaporation using a water bath (keeping the temperature below  $40^{\circ}\text{C}$ ). Suspend the resulting residue in DCM (55 ml) and wash with 20% aqueous KI ( $3 \times 55$  ml) and water (55 ml). Collect the organic fraction, dry with magnesium sulfate, remove the drying agent by filtration and remove the solvent *in vacuo* to afford a syrup. Dilute the syrup with ether (44 ml) and filter the solution through

a short pad of Florisil (100–200 mesh) to afford a colourless solution. After further washings of the Florisil with ethyl acetate (16 ml), combine the organic phases and remove the solvent *in vacuo* to yield the aldehyde (**42**) as an oil in 80% yield.  $^1\text{H}$  NMR (DMSO- $d_6$ , 180  $^\circ\text{C}$ ) 3.64 (dd, 1H,  $J = 6.4, 9.6$  Hz), 3.70 (dd, 1H,  $J = 5.3, 9.6$  Hz), 3.80–3.88 (m, 3H), 4.03 (t, 1H,  $J = 8.6$  Hz), 4.08 (dd, 1H,  $J \sim 0.8, 2.3$  Hz), 4.50 and 4.54 (2 d, 2H,  $J = 10.7$  Hz), 7.20–7.40 (m, 20H), 9.60 (d, 1H,  $J = 1.4$  Hz).

*Part 2: olefination of the aldehyde (42) to allow synthesis of (1,6)-C-disaccharides*



i) *n*-BuLi,  $-30^\circ\text{C}$ , THF-HMPA; ii)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2$ ; iii) Amberlite IR-120,  $\text{H}_2\text{O}$ .

*Notes and discussion.* Sugar aldehydes such as aldehyde (**42**) have proved of use for entry to C-linked glycosides via an olefination and reduction strategy. The pre-established axial or equatorial orientation of the formyl group allows entry to defined anomers of the C-linked glycosides. A range of (1,6)-linked C-di-, tri- and tetrasaccharides with both  $\alpha$ - and  $\beta$ -linkages have been accessed in this way.

#### Materials.

Aldehyde ( <b>42</b> ) (150 mg)	treat as harmful
Phosphonium sugar ( <b>43</b> ) [59] (350 mg, 0.55 mmol)	treat as harmful
HMPA (1 ml)	flammable, irritant
<i>n</i> -BuLi (346 $\mu\text{l}$ , 0.55 mmol, 1.6 M in hexane)	flammable, moisture sensitive
Anhydrous THF	flammable, irritant
Celite <sup>®</sup>	irritant
Diethyl ether	flammable, irritant

Water

Magnesium sulfate irritant

Cyclohexane and ethyl acetate for chromatography flammable,  
irritant

Ethanol highly flammable

$\text{Pd}(\text{OH})_2$  (20%) (20 mg) irritant, air  
sensitive

Activated 4 Å powdered molecular sieves (480 mg) irritant

Amberlite IR-120 ( $\text{H}^+$ ) ion-exchange resin irritant

### Equipment.

Round bottomed flasks with rubber septa plus magnetic stirrer bars

Magnetic stirrer

Source of dry nitrogen or argon  
– 30 °C cooling bath

Dry glass syringes and needles

Separatory funnel and conical flasks

Filter funnel and filter paper

Water bath

Rotary evaporator

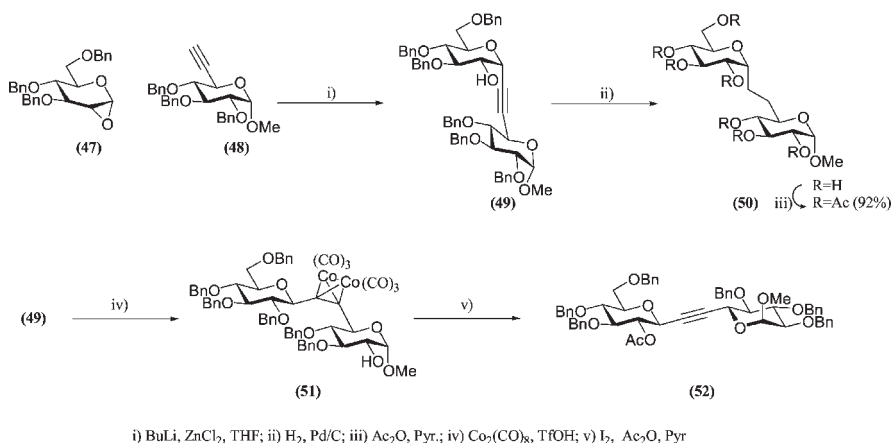
Equipment for chromatography

**Method.** All glassware must be dried in an oven prior to use, since each stage of the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard. Place the phosphonium salt (**43**) (350 mg, 0.55 mmol), activated 4 Å powdered molecular sieves (480 mg), anhydrous THF (2 ml), and HMPA (1 ml) into a dry round bottomed flask under an inert atmosphere, that should be maintained throughout the reaction. Cool the suspension to – 30 °C via external cooling. Add *n*-BuLi (346  $\mu\text{l}$ , 0.55 mmol of a 1.6 M solution in hexane), followed by 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-aldehydo-D-glycero-L-manno-heptopyranose (**42**) (150 mg) in anhydrous THF (1.2 ml). Allow the suspension to warm to room temperature over a period of 2.5 h, and leave the suspension stirring at this temperature for 30 min. Then filter the suspension through Celite<sup>®</sup>. Dilute the filtrate with diethyl ether (20 ml) and transfer the mixture to a separatory funnel. Wash the organic layer with water (10 ml), collect the organic fraction and dry with magnesium sulfate. Remove the drying agent by filtration, and remove the solvent from the filtrate *in vacuo*. Purification can be achieved by flash chromatography using silica gel and cyclohexane/ethyl acetate 4:1, as the eluent. The disaccharide (**44**) is isolated as an oil in 76% yield. Add ethyl acetate/ethanol (1:1, 5 ml) to the disaccharide (**44**) (111 mg, 0.14 mmol). Then add  $\text{Pd}(\text{OH})_2$  (20%, 20 mg) and stir the reaction mixture under an atmosphere of hydrogen for 4 h at a pressure of 3 atm.

Remove the catalyst by filtration and concentrate the filtrate *in vacuo*. Purify the residue using silica gel and ethyl acetate/methanol 9:1 as the eluent to afford (**45**) in 95% yield. Dissolve this residue in water (3 ml) and add Amberlite IR-120 (H<sup>+</sup>) ion-exchange resin. Heat the reaction mixture to 70 °C for 2 h. Cool the reaction mixture to room temperature then remove the resin by filtration and concentrate the filtrate *in vacuo* to afford the C-disaccharide (**46**) in 85% yield. Data for (**45**) [ $\alpha$ ]<sub>D</sub> = -38 (c 1.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CD<sub>3</sub>OD) 1.34, 1.43, 1.56, (3 × s, 2 × C(CH<sub>3</sub>)<sub>2</sub>, 12H), 1.57 (m, H-7', 1H), 1.70 (m, H-6', 1H), 1.80 (m, H-6, 1H), 2.14 (m, H-7, 1H), 3.10 (dt,  $J_{8,7'} = 2.0$  Hz,  $J_{8,7'} \approx J_{8,9} \approx 9.0$  Hz, H-8, 1H), 3.45 (t,  $J_{9,10} = 9.0$  Hz, H-9, 1H), 3.46 (dd,  $J_{10,11} = 3.5$  Hz, H-10, 1H), 3.47 (m, H-12, 1H), 3.70 (dd,  $J_{13,13'} = 12.0$  Hz,  $J_{13,12} = 5.5$  Hz, H-13, 1H), 3.72 (dd,  $J_{13',12} = 7.0$  Hz, H-13, 1H), 3.80 (ddd,  $J_{5,4} = 2.0$  Hz,  $J_{5,6} = 8.0$  Hz,  $J_{5,6'} = 5.5$  Hz, H-5, 1H), 3.92 (d, H-11, 1H), 4.20 (dd,  $J_{4,3} = 8.0$  Hz, H-4, 1H), 4.36 (dd,  $J_{2,3} = 2.5$  Hz,  $J_{2,1} = 5.0$  Hz, H-2, 1H), 4.62 (dd, H-3, 1H), 5.48 (d, H-1, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) 22.0, 22.7, 23.8, 23.9, 25.0, 27.0, 60.2, 66.8, 68.3, 69.4, 69.7, 70.3, 71.5, 73.9, 77.6, 79.0, 95.4, 107.1, 107.5. Anal. Calcd for C<sub>19</sub>H<sub>32</sub>O<sub>10</sub>: C, 54.27, H, 7.67. Found: C, 54.41, H, 7.81.

### 11.2.4 1,2-Anhydro sugars as electrophilic donors

Early work by Danishefsky *et al.* [63] saw the development of 1,2-anhydro sugars (glycals) as key intermediates in the synthesis of β-linked oligosaccharides. This methodology was later developed to allow the generation of β-mannosides [64] which are common in nature yet difficult to synthesise due to the disfavoured 1,2-*cis* relationship (See Chapter 8). van Boom and co-workers recently employed this methodology for the synthesis of C-linked glycosides (Scheme 11.12) [65]. Stereoselective ring opening of the 1,2-anhydrosugar (**47**) with the lithiated alkynyl



Scheme 11.12

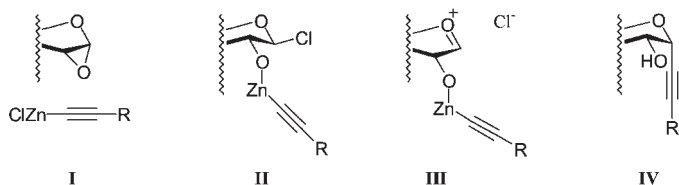


Figure 11.5

derivative (**48**) (or other suitable alkynes), in the presence of  $\text{ZnCl}_2$ , affords the  $\alpha$ -C-(alkynyl)-glycoside (**49**).

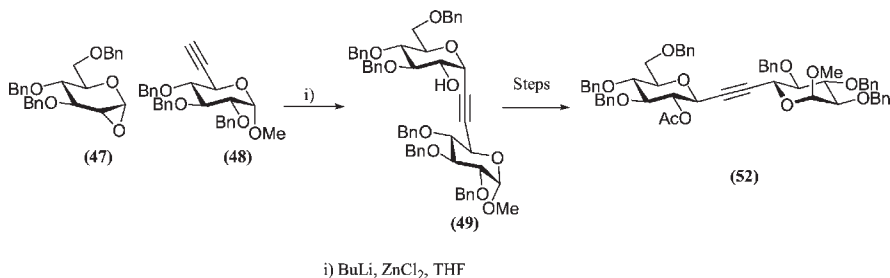
The unexpected stereochemical outcome is rationalised (Figure 11.5) by the generation of an initial zinc acetylide species which facilitates intramolecular delivery to the  $\alpha$ -face.

Of equal significance is the ability to control the anomeric stereochemistry. Isomerisation can be initiated by protection of the alkyne as the dicobalthexacarbonyl complex (**51**); subsequent epimerisation with catalytic amounts of triflic acid affords the  $\beta$ -anomer (**52**).

More recent work by van Boom and co-workers has seen the use of this method in the synthesis of *cis*- and *trans*-fused bicyclic ethers via a ring closing metathesis reaction [66].

### Method 6

Stereoselective synthesis of  $\alpha$ -C-(alkynyl)-glycosides via ring opening of  $\alpha$ -1,2-anhydrosugars [65, 66].



**Notes and discussion.** Ring-opening of an  $\alpha$ -1,2-epoxide function in sugars with lithium alkynyl derivatives, in the presence of zinc chloride, has been shown to proceed with retention of configuration to afford  $\alpha$ -C-alkynyl glycosides in good yields.

### Materials.

1,2-Anhydro-3,4,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranose (**47**) [67]      treat as harmful (0.6 equiv.)

Fully benzylated methyl 6,7-dideoxy- $\alpha$ -D-glucopyranoside (**48**) [68] (1 equiv.)

Dry tetrahydrofuran (0.7 ml/mmol)	flammable, irritant treat as harmful
<i>n</i> -Butyllithium (1 equiv.)	flammable, corrosive, moisture sensitive
ZnCl <sub>2</sub> (1 M solution in THF)	corrosive, hygroscopic
Saturated aqueous ammonium chloride	harmful, irritant
Ether	flammable, irritant
Brine	treat as harmful
Magnesium sulfate	irritant
Hexane and petroleum ether for chromatography	flammable, irritant
Co <sub>2</sub> (CO) <sub>8</sub> (1.1 equiv.)	toxic, avoid contact
Triflic acid (0.1 equiv.)	irritant
Saturated sodium hydrogen carbonate	treat as harmful
Iodine (5 equiv.)	harmful
Aqueous 10% sodium thiosulfate solution	irritant

*Equipment.*

Round-bottomed flasks (various sizes) with rubber septa plus magnetic stirrer bars  
Magnetic stirrer  
Source of dry nitrogen or argon  
Source of dry air  
– 78 °C cooling bath  
Separatory funnel and conical flasks  
Filter funnel and filter paper  
Rotary evaporator  
Equipment for chromatography

*Procedure.* All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.



*For entry to the  $\alpha$ -linked C-disaccharide:* Equip a dry round bottomed flask with a magnetic stirrer bar and rubber septum, and establish an inert atmosphere, that must be maintained throughout the reaction. Add acetylene (**48**), and dry THF (0.7 ml/mmol) to the flask and cool to  $-78^\circ\text{C}$ . Next add *n*-BuLi (1 equiv.) and leave to stir for 30 min. Add the  $\alpha$ -1,2-anhydro sugar (**47**) (0.6 equiv.) in THF (1 ml/mmol) and then a 1 M solution of  $\text{ZnCl}_2$  in THF. Warm the mixture to room temperature and leave the mixture to stir for a further 1 h. Quench the reaction by the addition of saturated aqueous ammonium chloride, transfer the mixture to a separatory funnel and extract the aqueous layer using diethyl ether. Collect the organic fractions and wash with brine, dry the organic layers with magnesium sulfate, remove the drying agent by filtration, and remove the solvent *in vacuo* to afford the crude  $\alpha$ -product. Purification can be performed by column chromatography using silica gel and ethyl acetate/light petroleum to afford the C-disaccharide (**49**) in 59% yield.

*For entry to the  $\beta$ -linked C-disaccharide:* Add  $\text{Co}_2(\text{CO})_8$  (1.1 equiv.) to the  $\alpha$ -isomer (**49**) in DCM (4 ml/mmol) under an inert atmosphere. Leave the reaction to stir at room temperature for 1.5 h. In order to ensure complete oxidation of excess reagent air should then be bubbled through the reaction mixture for 15 min. Then remove the solvent *in vacuo*. Purification is achieved via flash chromatography, using ethyl acetate/light petroleum. Redissolve the resulting dark red complex in an equivalent volume of dry DCM, under an inert atmosphere, and add triflic acid (0.1 equiv.) under a stream of nitrogen. TLC analysis can be used to follow the course of the reaction. When the reaction is shown to be complete by TLC analysis, add saturated sodium hydrogen carbonate and diethyl ether and transfer the mixture to a separatory funnel. Wash the mixture with water and brine. Collect the organic fractions and dry them with magnesium sulfate, remove the drying agent by filtration and remove the solvent from the filtrate *in vacuo*. Dissolve the crude reaction mixture in an equivalent volume of THF. Add  $\text{I}_2$  (5 equiv.) and allow the solution to stir at room temperature for 3 h. After this time add a 10% solution of  $\text{Na}_2\text{SO}_3$  followed by saturated sodium hydrogen carbonate and diethyl ether. Separate the layers and collect the organic fraction. Wash the organic fraction with a 10% solution of  $\text{Na}_2\text{SO}_3$  and brine. Dry the organic layer with magnesium sulfate, remove the drying agent by filtration and concentrate the filtrate *in vacuo*. The residue can be purified by column chromatography using silica gel and ethyl acetate/light petroleum to afford the  $\beta$ -anomer (**52**) in 66% yield.

Selected data for **49**;  $R_f$  0.57 (50% EtOAc/light petroleum);  $m/z$  (ESI) 909 ( $\text{M} + \text{NH}_4$ )<sup>+</sup>, 914 ( $\text{M} + \text{Na}$ )<sup>+</sup>.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (for acetylated derivative) 7.37–7.10 (m, ArCH, 30H), 5.07 (dd,  $J = 1.6, 6.0$  Hz, H-1', 1H), 5.00–4.44 (m, BnCH<sub>2</sub>, 12H), 4.92 (dd,  $J = 9.9$  Hz, H-2', 1H), 4.54 (d,  $J = 3.1$  Hz, H-1, 1H), 4.42 (dd,  $J = 9.8$  Hz, H-5, 1H), 3.98 (m, H-5', 1H), 3.74 (dd, H-6<sub>A</sub>, 1H), 3.68 (dd, H-4', 1H), 3.64 (dd, H-6<sub>B</sub>, 1H), 3.51 (m, H-2, H-4, 2H), 3.41 (s, OCH<sub>3</sub>, 3H), 1.91 (s, OAc, 3H).

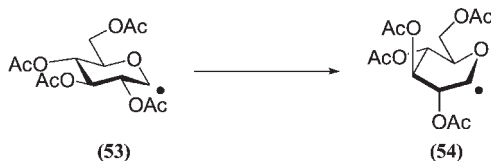
*The radical approach [69]*

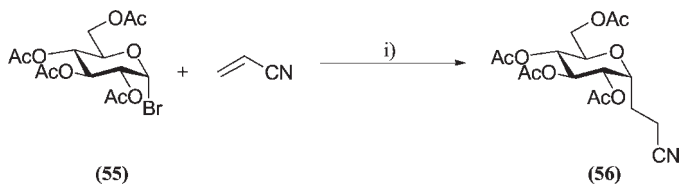
The radical approach for C–C bond formation is a popular method within organic chemistry. The use of radical chemistry in carbohydrate synthesis has certain advantages. Firstly the reaction conditions are very mild and tolerant of a range of functional and protecting groups. Anomeric radicals are also stable towards elimination and epimerisation. Most significantly, the chemistry required to incorporate an appropriate substituent at C-1, employed in the initial homolytic cleavage step, is common within carbohydrate chemistry. The use of such radical techniques can be subdivided into two classes, intermolecular and intramolecular reactions.

**11.2.5 Intermolecular radical reactions**

Giese has extensively investigated the area of radical chemistry [70] and in 1983 he applied this knowledge to the synthesis of C-glycosides [71]. As a result, it was concluded that the addition of an alkene to an anomeric radical, under appropriate conditions, allowed exclusive entry to the  $\alpha$ -linked C-glycoside, a result which was mirrored by the work of Baldwin [72]. The high  $\alpha$ -selectivity of the reaction was in contrast with other reports. Thus radicals generated at the C-2, C-3 and C-4 centres all preferred to occupy equatorial positions [73]. The initial explanation for this stereochemical preference was that the anomeric radical preferentially adopted the axial orientation which enables an interaction with the non-bonding electron pair on the ring oxygen, and thus led to its stabilisation. However, subsequent work by Giese [70, 74] discovered that upon generation of the anomeric radical, a conformational change also occurs such that the preferred  ${}^4C_1$  chair conformation of the starting material (**53**) is replaced by the observed  $B_{2,5}$  conformation (**54**) in the intermediate (Scheme 11.13).

This change is rationalised by the resulting interaction between the SOMO of the radical and the LUMO of the adjacent C–OAc bond. Radical attack can then occur from the favoured quasi-equatorial face, and conversion to the original chair conformation generates the axially substituted compound. Further evidence in favour of the transformation can be observed in the mannose series where no such

**Scheme 11.13**



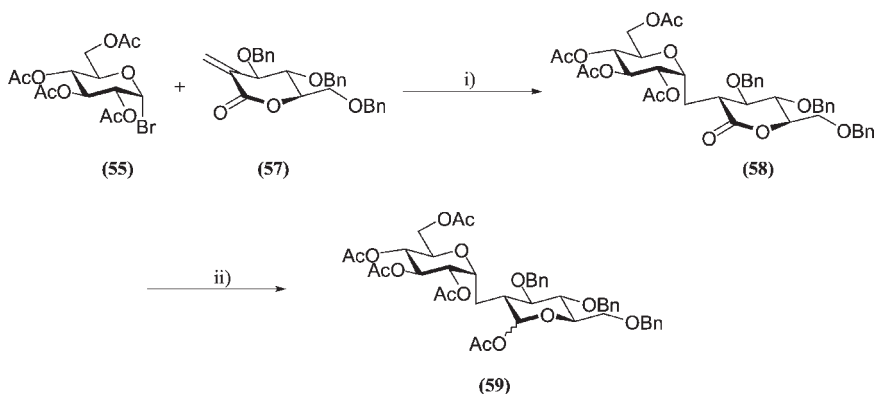
i)  $\text{Bu}_3\text{SnH}$ ,  $h\nu$ , ether, reflux.

**Scheme 11.14**

conformational change is observed since the C-2 substituent already resides in the favoured axial position.

Reaction of 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide (**55**) with tributyltin hydride and acrylonitrile under photolytic conditions afforded the  $\alpha$ -glycoside (**56**) in 75% yield with an  $\alpha/\beta$  ratio of 93:7 (Scheme 11.14).

The work has been extended to incorporate a range of sugars, including galactose and mannose, and a range of substituted alkenes, including fumarodinitrile and methacrylonitrile. All reactions afforded the  $\alpha$ -glycosides in good yield and with good selectivities. Further work has seen the generation of *C*-disaccharides [75] via radical C–C bond formation, using carbohydrate derived alkenes such as (**57**) (Scheme 11.15). In addition, hydrogen abstraction at the resulting radical centre also occurs from the axial face to selectively generate the equatorial C-2 substituent in the resulting disaccharide (**58**).

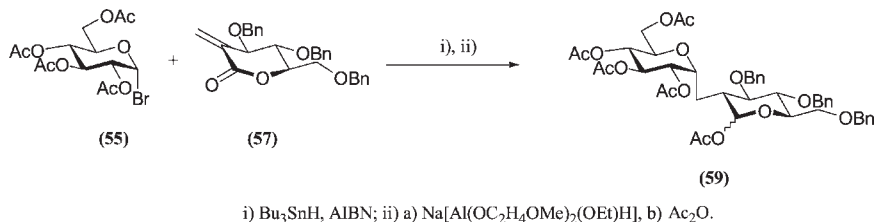


i)  $\text{Bu}_3\text{SnH}$ , AIBN; ii) a)  $\text{Na}[\text{Al}(\text{OC}_2\text{H}_4\text{OMe})_2(\text{OEt})\text{H}]$ , b)  $\text{Ac}_2\text{O}$ .

**Scheme 11.15**

*Method 7*

Synthesis of *C*-disaccharides by reaction of carbohydrate anomeric radicals to carbohydrate derived alkenes [75]



*Notes and discussion.* Access to *C*-disaccharides in which the pyranosyl rings are linked via a methylene group is described. In order to determine the stereochemistry at C-1' of the acetylated derivative (59),  $^1\text{H}$  NMR spectra were recorded at several temperatures. A doublet for H-1' ( $J = 5.1$  Hz) was observed at 20 °C but this signal was split at  $-80$  °C to give a doublet with a coupling constant of 8.5 Hz and a broad singlet. Such splittings indicate the *trans*-geometry of the substituents at C-1' and C-2'. Thus at  $-80$  °C, the ratio of (59a):(59b) was determined as 70:30.

*Materials.*

Tetra-*O*-acetylglucosyl bromide (55) [76]

treat as harmful

Lactone (57) [77]

Anhydrous ether (2 ml/mmol)

flammable, irritant

Acrylonitrile (5 + 2.4 equiv.)

highly  
flammable,  
possible  
carcinogen

Tributyltin hydride (1.1 + 0.4 equiv.)

toxic, freeze, air  
sensitive

Acetonitrile

toxic

Pentane

highly flammable

Ethyl acetate and hexane for flash chromatography

flammable, irritant

*Equipment.*

Two-necked round-bottomed flask with rubber septum in one neck and with water condenser (with rubber septum) in the second neck, plus magnetic stirrer bar

Magnetic hotplate stirrer with silicone oil heating bath and temperature probe

Source of dry nitrogen or argon

Dry glass syringe

Sun lamp or a high-pressure mercury lamp

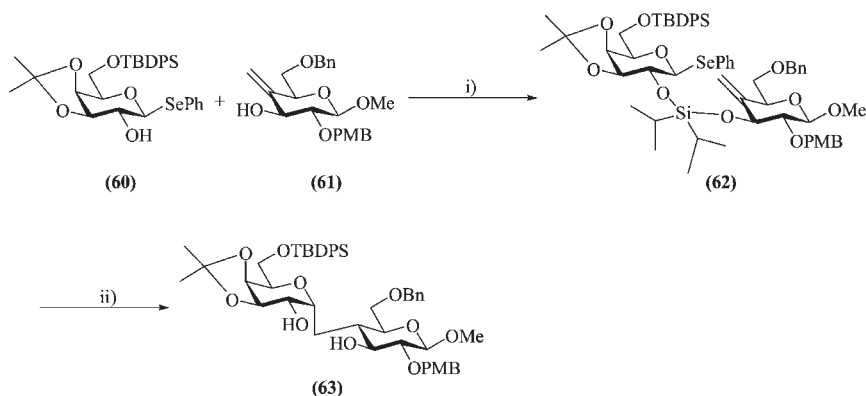
Separatory funnel and conical flasks  
Rotary evaporator  
Equipment for chromatography

*Procedure.* All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard.

Equip a dry, two necked flask with a water condenser (topped with a rubber septum), a magnetic stirrer bar and rubber septum and establish an inert atmosphere that must be maintained throughout the reaction. Add bromide (**55**) and anhydrous ether (2 ml/mmol) to the flask and heat to reflux. Next add acrylonitrile (5 equiv.) and tributyltin hydride (1.1 equiv.) and irradiate with a sun lamp or a high-pressure mercury lamp for 4 h. Filter the resulting precipitate and add further acrylonitrile (2.4 equiv.) and tributyltin hydride (0.4 equiv.) and subject the filtrate to further irradiation. Once TLC analysis confirms the reaction to be complete cool and filter the reaction. Combine the precipitates and recrystallise from ether to afford the  $\alpha$ -glycoside (**58**). Concentrate the remaining filtrate *in vacuo* and redissolve the residue in acetonitrile. Extract the solution three times with pentane. Remove the acetonitrile layer and remove the acetonitrile *in vacuo* to afford a syrup that can be purified by column chromatography using silica gel and hexane/ethyl acetate 1:1, as the eluent to afford a further amount of glycoside (**58**). [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +66.2 (*c* 0.7, CHCl<sub>3</sub>), IR (KBr) 2240 (C≡N), 1745 (C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub> numbering from C≡N as C=1) 1.84–1.95 (m, H-3a, 1H), 2.05 (s, OAc, 6H), 2.09 (s, OAc, 3H), 2.11 (s, OAc, 3H), 2.05–2.24 (m, H-3b, 1H), 3.46 (m, H<sub>2</sub>-2, 2H), 3.88 (ddd, *J* = 8.3, 2.9, 5.8 Hz, H-8, 1H), 4.12 (dd, *J* = 5.8, 12.2 Hz, H-9a, 1H), 4.23 (m, H-4, 1H), 4.32 (dd, *J* = 5.8, 12.2 Hz, H-9b, 1H), 4.98 (t, *J* = 8.3 Hz, H-7, 1H), 5.09 (dd, *J* = 5.2, 8.3 Hz, H-5, 1H), 5.23 (t, *J* = 8.3 Hz, H-6, 1H); Anal. Calc. for C<sub>17</sub>H<sub>23</sub>NO<sub>9</sub>: C, 52.98; H, 6.02; N, 3.63. Found: C, 52.80; H, 6.01; N, 3.63.

### 11.2.6 Intramolecular radical reactions

One disadvantage with the aforementioned intermolecular approaches is that during such radical mediated reactions, electron deficient alkenes are required to facilitate C–C bond formation. The intramolecular approach of Sinaÿ overcomes this drawback by bringing together the two substrates via a temporary covalent silaketal connector (Scheme 11.16). This enables the use of a wider range of alkenes, which can include alkene functionalised sugars, thus leading to the generation of C-linked disaccharides. Developing the early work of Stork [78], Sinaÿ employed the tether reaction in the synthesis of both  $\alpha$ - and  $\beta$ -C-disaccharides [79], with this work subsequently being employed in the synthesis of biologically significant natural products [80]. The availability of different hydroxyl

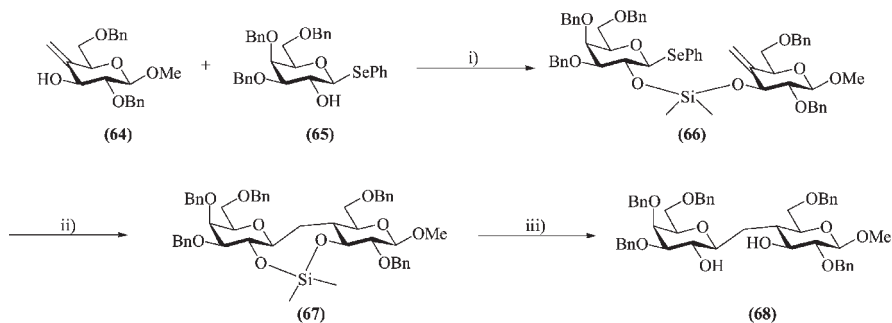


i) a)  $n\text{-BuLi}$ , THF,  $0^\circ\text{C}$ , b)  $i\text{Pr}_2\text{SiCl}_2$ , 4 equiv.,  $-78^\circ\text{C}$  to r.t. c) Concentrate to dryness, d) (61), DMAP, THF, r.t.; ii)  $\text{Bu}_3\text{SnH}$ , AIBN, toluene, reflux.

**Scheme 11.16**

groups on each monosaccharide unit allowed the fine-tuning of the stereochemical outcome by selective positioning of the silyl tether [81]. Development of this early stereochemical control led to the tethering of donor (61) with alcohol (60) through a di-isopropyl silicon tether (62). Subsequent treatment with  $\text{Bu}_3\text{SnH}$  and AIBN, led to a 9-*endo*-trig cyclisation to form the C-disaccharide (63) in an 80% yield with exclusive formation of the  $\alpha$ -product (Scheme 11.16).

In parallel, the development of a 3,2' silyl tether allowed access to the  $\beta$ -linked product as demonstrated in Sinäy's synthesis of methyl  $\beta$ -C-lactoside (68) (Scheme 11.17) [82]. Alcohols (64) and (65) were connected via



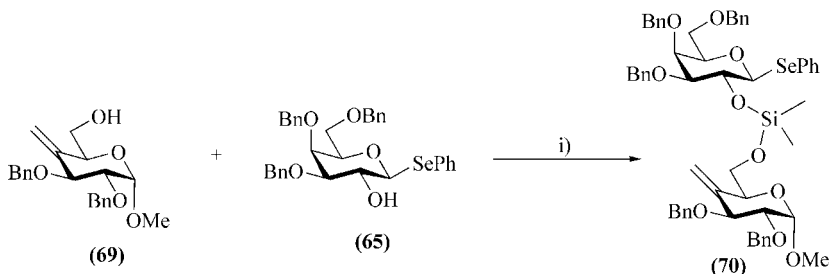
i) a)  $\text{PhSeH}$ ,  $\text{Me}_2\text{SiCl}_2$  4.4 equiv.,  $\text{BuLi}$ , THF,  $-78^\circ\text{C}$  to r.t., b) Alkene, imidazole, THF, r.t.; ii)  $\text{Bu}_3\text{SnH}$ , AIBN, toluene,  $110^\circ\text{C}$ , 17h; iii)  $\text{Bu}_4\text{NF}$ , THF, r.t.

**Scheme 11.17**

This work has been developed to incorporate a range of substrates [83], and alternative tethering reagents [84], whilst maintaining the initial, excellent stereochemical control.

i) a) (65),  $\text{Me}_2\text{SiCl}_2$  4.4 equiv., BuLi, THF,  $-78^\circ\text{C}$  to r.t., b) Alkene, imidazole, THF, r.t.;  
ii)  $\text{Bu}_3\text{SnH}$ , AIBN, toluene,  $110^\circ\text{C}$ , 17h; iii)  $\text{Bu}_4\text{NF}$ , THF, r.t.

*Notes and discussion.* Selenophenyl glycosides are well established derivatives that are often crystalline. In this work their potential to afford anomeric radicals is exemplified. The donor and acceptor are joined together via a silaketal tether before radical formation is initiated. It is not generally necessary to purify the silaketal prior to radical formation. The  $\text{Bu}_3\text{SnH}$  and AIBN must be added very slowly to the silaketal, via syringe pump, for efficient reaction. Although a mixture of C-disaccharides results in this procedure, they can be separated to afford analytically pure material by careful chromatography.

*Part 1: formation of the silaketal tethered derivative (70)*

i) a) (65),  $\text{Me}_2\text{SiCl}_2$ , 4.4 equiv., BuLi, THF,  $-78^\circ\text{C}$  to r.t., b) (69), imidazole, THF, r.t.

*Materials.*

Phenyl selenide (65) [84] (1.7 g, 2.88 mmol)

assume toxic

Dry THF

irritant,  
flammable

*n*-BuLi (1.6 M solution in hexane, 2.34 ml, 3.74 mmol)

toxic, moisture  
sensitive

Dichlorodimethylsilane (2.22 ml, 10.1 mmol)

toxic, moisture  
sensitive

Imidazole (0.30 g, 4.35 mmol)

toxic

Alkene (69) [84] (1.077 g, 2.9 mmol)

assume toxic

Dichloromethane

toxic, irritant

Magnesium sulfate

irritant

Cyclohexane and ethyl acetate and  $\text{Et}_3\text{N}$  for chromatography

flammable,  
irritant

*Equipment.*

Schlenk tubes

Magnetic stirrer

Source of dry nitrogen or argon

–  $78^\circ\text{C}$  cooling bath

Dry glass syringes with needles

Separatory funnel and conical flasks

Glass sinter funnel

Rotary evaporator

High vacuum

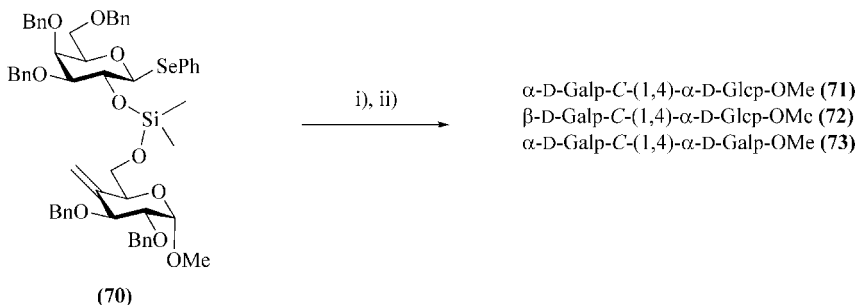
Equipment for chromatography

All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses



should be worn during the experiment, and all manipulations should be performed in a fume cupboard. Add phenyl selenide (**65**) (1.7 g, 2.88 mmol) to a dry Schlenk tube and establish an inert atmosphere. Then add dry THF (6 ml) and cool the solution to  $-78^{\circ}\text{C}$ . Add *n*-BuLi (1.6 M solution in hexane, 2.34 ml, 3.74 mmol) with stirring and leave the solution to stir for 15 min at  $-78^{\circ}\text{C}$ . After 15 min, add dichlorodimethylsilane (2.22 ml, 10.1 mmol) and stir the solution for a further 30 min at  $-78^{\circ}\text{C}$  and then for 3 h at room temperature. Remove the solvents from the Schlenk tube under high vacuum and dissolve the residue in dry THF (2 ml). Add a solution of the alkene (**69**) (1.077 g, 2.9 mmol) and imidazole (0.30 g, 4.35 mmol), in dry THF (6 ml) to the Schlenk tube, at room temperature, with stirring. Continue stirring for 1 h and then add dichloromethane and water. Transfer the mixture to a separatory funnel and extract the aqueous layer with dichloromethane, combine the organic fractions, dry with magnesium sulfate, remove the drying agent by filtration, and concentrate the filtrate *in vacuo*. In general the silaketal is used immediately for the cyclisation reaction, but if desired, it can be purified by flash column chromatography on silica gel using cyclohexane/ethyl acetate 6:1 to 4:1 in the presence of  $\text{Et}_3\text{N}$  to afford pure silaketal (**70**) in 87% as a syrup.  $^1\text{H}$  NMR (400 MHz,  $\text{C}_6\text{D}_6$ ) 7.96–7.07 (m, 30H, Ph), 5.65 (s, 1H, HC=), 5.22 (s, 1H, HC=), 4.98 (d, 1H,  $J_{1'2'} = 9.5$  Hz, H-1'), 4.95 and 4.57 (ABq, 2H,  $J = 11.5$  Hz,  $\text{PhCH}_2$ ), 4.86 (d, 1H,  $J_{1,2} = 3.3$  Hz, H-1), 4.81 and 4.71 (ABq, 2H,  $J = 11.7$  Hz,  $\text{PhCH}_2$ ), 4.73 and 4.55 (ABq, 2H,  $J = 12.0$  Hz,  $\text{PhCH}_2$ ), 4.65 (dd, 1H,  $J_{2',3'} = 9.5$  Hz, H-2'), 4.61 (d, 1H,  $J_{3,4} = 9.0$  Hz, H-3), 4.52–4.45 (m, 3H, H-5 and  $\text{PhCH}_2$ ), 4.53 (dd, 1H,  $J_{5,6a} = 5.7$  Hz,  $J_{6a,6b} = 10.5$  Hz, H-6a), 4.35 and 4.28 (ABq, 2H,  $J = 11.8$  Hz,  $\text{PhCH}_2$ ), 4.33 (dd, 1H,  $J_{5,6b} = 5.6$  Hz, H-6b), 3.94 (d, 1H,  $J_{3',4'} = 2.5$  Hz, H-4'), 3.78 (dd, 1H,  $J_{5',6'a} = 7.5$  Hz,  $J_{6'a,6'b} = 8.5$  Hz, H-6'a), 3.70 (dd, 1H,  $J_{5,6'b} = 6.5$  Hz, H-6'b), 3.69 (dd, 1H,  $J_{2,3} = 9.0$  Hz, H-2), 3.51 (dd, 1H, H-5'), 3.34 (s, 3H,  $\text{OCH}_3$ ), 3.30 (dd, 1H, H-3'), 0.35 and 0.40 (2 s, 6H, Me-Si). Prime refers to the selenophenyl unit.  $m/z$  (CI) 1034 ( $\text{M} + 18$ ).

*Part 2: radical addition reaction to afford the C-linked saccharides (71), (72) and (73)*



i)  $\text{Bu}_3\text{SnH}$ , AIBN, toluene,  $110^{\circ}\text{C}$ , 17h; ii)  $\text{Bu}_4\text{NF}$ , THF, r.t.

*Materials.*

Silaketol ( <b>70</b> ) (0.68 mmol)	assume toxic
Dry toluene	irritant, flammable
Bu <sub>3</sub> SnH (403 $\mu$ l, 1.5 mmol)	toxic, freeze, air sensitive
AIBN (11.2 mg)	toxic, moisture sensitive
THF	toxic, flammable
40% aqueous HF (1.5 ml)	assume toxic
Saturated NaHCO <sub>3</sub>	assume toxic, irritant
Magnesium sulfate	irritant
Water	
Dichloromethane	toxic, irritant
Cyclohexane and ethyl acetate for chromatography	flammable, irritant

*Equipment.*

Two necked flask equipped with a reflux condensor, rubber septa and magnetic stirring bar  
Round bottomed flask equipped with a rubber septum and magnetic stirrer bar  
Source of dry nitrogen or argon  
Syringe pump  
Hot plate stirrer  
Dry glass syringes with needles and syringe pump  
Separatory funnel and conical flasks  
Filter funnel and filter paper  
Rotary evaporator  
Equipment for chromatography

All glassware must be dried in an oven prior to use, since the reaction must be performed under anhydrous conditions, in an inert atmosphere. Safety glasses should be worn during the experiment, and all manipulations should be performed in a fume cupboard. For a typical reaction, transfer the silaketol (**70**) (0.68 mmol) to a two-necked flask equipped with a reflux condensor. Establish inert conditions and add anhydrous toluene (34 ml). Degass the solution three times to ensure dry and oxygen free conditions. Heat the solution to reflux. Prepare a solution of Bu<sub>3</sub>SnH (403  $\mu$ l, 1.5 mmol) and AIBN (11.2 mg) in anhydrous toluene (34 ml) and again

degass this solution three times. Transfer this solution to a syringe pump and add, during 15 h, to the solution of silaketal (**70**) at reflux in toluene. After this time, cool the solution and remove the solvent *in vacuo*. Redissolve the residue in THF (10 ml) and add 40% aqueous HF (1.5 ml) and stir the solution for 2 h. Then neutralize the solution with aqueous saturated  $\text{NaHCO}_3$ , dilute the solution with water and transfer the mixture to a separatory funnel. Extract the aqueous layer with dichloromethane ( $3 \times 50$  ml). Combine the organic layers, dry with magnesium sulfate, remove the drying agent by filtration, and concentrate the filtrate *in vacuo*. Purification of the residue by flash column chromatography (cyclohexane/ethyl acetate 1:1 to ethyl acetate) affords first 1,5-anhydro-3,4,6-tri-*O*-benzyl-D-galactitol (111 mg, 37%) as a syrup, then recovered alkene (**69**) (76.5 mg, 40%), then a co-eluting mixture of the 3 *C*-disaccharides (**71**), (**72**) and (**73**) (328 mg, 60%). The ratio of these saccharides was evaluated by NMR and determined to be **71**:**72**:**73** of 65:25:10. After further chromatography using the same system, Compounds **71** and **72** are obtained as pure fractions.

Compound **71**: syrup,  $[\alpha]_D = +55$  (*c* 1.0,  $\text{CHCl}_3$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{C}_6\text{D}_6$ ) 7.41–7.23 (m, 25H, Ph), 5.10 and 4.71 (ABq, 2H,  $J = 11.5$  Hz,  $\text{PhCH}_2$ ), 4.77 and 4.68 (ABq, 2H,  $J = 12.0$  Hz,  $\text{PhCH}_2$ ), 4.75 (d, 1H,  $J_{1,2} = 3.5$  Hz, H-1), 4.67 and 4.57 (ABq,  $J = 11.5$  Hz,  $\text{PhCH}_2$ ), 4.62 and 4.58 (ABq, 2H,  $J = 12.5$  Hz,  $\text{PhCH}_2$ ), 4.58 and 4.51 (ABq, 2H,  $J = 12.0$  Hz,  $\text{PhCH}_2$ ), 4.23 (ddd, 1H,  $J_{4'5'} = 5.5$  Hz,  $J_{5'6'a} = 10.0$  Hz,  $J_{5'6'b} = 3.0$  Hz, H-5'), 4.16 (d, 1H,  $J_{6'a6'b} = 10$  Hz, H-6a'), 4.13–4.06 (m, 1H, H-1'), 3.95 (dd, 1H,  $J_{3'4'} = 3.0$  Hz, H-4'), 3.92–3.85 (m, 1H, H-6a), 3.75 (dd, 1H,  $J_{2'3'} = 5.0$  Hz, H-3'), 3.72–3.66 (m, 3H, H-2', H-3, H-6b), 3.60 (dd, 1H,  $J_{2,3} = 9.0$  Hz, H-2), 3.58 (dd, 1H, H-6'b), 3.49–3.43 (m, 1H, H-5), 3.42 (s, 3H,  $\text{CH}_3\text{O}$ ), 3.33–3.25 (m, 1H, OH-6), 2.0–1.9 (m, 1H, H-4), 1.58–1.43 (m, 2H, H-4 $\alpha$ , H-4a', methylene bridge);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) 138.7–127.3 (30C, Ph), 98.3 ( $\text{OCH}_3$ ), 81.4, 80.7, 76.6, 73.7, 72.8, 72.6, 70.1, 69.9 (8C), 75.3, 73.1, 72.8, 72.6, 71.7 (5 $\text{PhCH}_2$ ), 65.3, 61.9 (C6, C6'), 55.1 (OMe), 39.6 (C4), 28.6 (C4 $\alpha$ , methylene bridge);  $m/z$  (CI) 822 ( $\text{M}^+ + 18$ ), 773 ( $\text{M}^+ - \text{OCH}_3$ ).

Compound **72**: syrup,  $[\alpha]_D = +25$  (*c* 1.6,  $\text{CHCl}_3$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{C}_6\text{D}_6$ ) 7.27–7.47 (m, 25H, Ph), 5.17 and 4.70 (ABq, 2H,  $J = 11.0$  Hz,  $\text{PhCH}_2$ ), 4.92 and 4.65 (ABq, 2H,  $J = 12.0$  Hz,  $\text{PhCH}_2$ ), 4.81 and 4.71 (ABq, 2H,  $J = 12.0$  Hz,  $\text{PhCH}_2$ ), 4.74 (d, 1H,  $J_{1,2} = 3.5$  Hz, H-1), 4.73 and 4.64 (ABq, 2H,  $J = 11.0$  Hz,  $\text{PhCH}_2$ ), 4.43 and 4.36 (ABq, 2H,  $J = 11.5$  Hz,  $\text{PhCH}_2$ ), 4.05 (ddd, 1H,  $J_{1'2'} = J_{2'3'} = 9.0$  Hz,  $J_{2',\text{OH}} = 3.0$  Hz, H-2'), 3.91 (d, 1H,  $J_{3',4'} = 2.7$  Hz,  $J_{4'5'} < 1.0$  Hz H-4), 3.88–3.84 (m, 1H, H-6a), 3.77 (dd, 1H,  $J_{2,3} = J_{3,4} 9.0$  Hz, H-3), 3.75–3.70 (m, 1H, H-6b), 3.67 (dd, 1H, H-2), 3.59–3.48 (m, 3H, H-5, H-5', H-6'a), 3.44–3.35 (m, 3H, H-1, H-3', H-6'b), 3.40 (s, 3H,  $\text{CH}_3\text{O}$ ), 3.27–3.21 (m, 1H, OH-6), 2.92 (d, 1H, OH-2'), 2.08 (dddd, 1H,  $J_{4,5} = 9.0$  Hz,  $J_{4,4\alpha} = J_{4,4\alpha'} 3.5$  Hz, H-4), 1.89–1.84 (m, 2H, H-4 $\alpha$  and H-4 $\alpha'$ , methylene bridge);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) 138.3–127.5 (30C, Ph), 98.2 (C-1), 82.9, 81.6, 79.3, 78.8, 77.3, 73.2, 72.9, 68.9 (8C, rings), 75.8, 74.1, 73.3, 72.7, 72.4 (5 $\text{PhCH}_2$ ), 72.4, 68.9 (C6), 55.0 (OMe), 36.7 (C4), 28.2 (C4 $\alpha$ , methylene bridge);  $m/z$  (CI) 822 ( $\text{M}^+ + 18$ ), 773 ( $\text{M}^+ - \text{OCH}_3$ ).

### 11.3 CONCLUSION

In this chapter, the biological importance of C-glycosides as subunits of natural products and as a new generation of carbohydrate based therapeutics has been illustrated. Methods are available in the literature for the synthesis of structurally simple C-glycosides as well as di- and larger saccharides containing C-linked subunits. As with traditional saccharide synthesis, it has been necessary to develop specific methods to allow regio- and stereoselective entry to the desired targets, and examples of representative examples have been described herein.

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## The Uses of Glycoprocessing Enzymes in Synthesis

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### 12.1 INTRODUCTION

The chemical formation of a particular glycosidic bond between, for example, two monosaccharides involves the consideration of three factors:

(i) *Reactivity*: Firstly, the glycosyl donor needs to be sufficiently reactive to form the new glycosidic bond. i.e. a good leaving group must be present at the anomeric centre.

(ii) *Regioselectivity*: Only one of the hydroxyl groups in the glycosyl acceptor should act as a nucleophile, otherwise a mixture of disaccharides will result. Furthermore, the hydroxyl groups in the glycosyl donor must be prevented from reacting with another molecule of itself to form oligomers of repeating glycosyl donor units. These requirements usually necessitate a number of reactions to protect both the donor and acceptor molecules. For disaccharide formation this usually requires the protection of all of the hydroxyl groups in the glycosyl donor and all except one in the glycosyl acceptor. This necessitates protection steps before the coupling of the donor to acceptor can be attempted and, of course, these groups also have to be removed at the end of the synthetic sequence. Clearly, the complexity of these additional steps is further increased when oligosaccharides containing more saccharide units are to be coupled.

(iii) *Stereoselectivity*: The configuration ( $\alpha$  or  $\beta$ ) of the stereogenic centre at the anomeric carbon of what was the glycosyl donor unit must also be controlled. Despite the development of a number of chemical techniques that allow partial control of the configuration at the anomeric centre, these methods are rarely general.



As a result mixtures of products are often formed that require careful purification; side products clearly also lower the yield of the desired product.

In order to solve some of the problems that face chemical approaches to glycosylation, a leaf can be taken from Nature's book. The existence of a vast number of different oligosaccharides within natural systems implies that biological systems solve these problems efficiently. Nature, however, does this without the use of protecting groups. Evolutionary changes over many millions of years have led to the existence of many families of enzymes, termed glycoprocessing or carbohydrate-processing enzymes. These are powerful catalysts that are very well adapted to these functions. Two main classes exist: glycosyltransferases (Gly-Ts), which construct glycosidic bonds, and glycosidases (or glycosylhydrolases), which cleave them. Despite their opposing functions there are sufficient similarities in the mechanism of Gly-Ts and glycosidases for both classes to be exploited successfully in synthesis.

(i) *Reactivity*: As for most chemical methods of forming glycosidic bonds, enzymatic mechanisms also rely on the loss of a good leaving group from the anomeric centre of the glycosyl donor. For glycosidases this is often either an existing good-leaving group (e.g. *para*-nitrophenyl or fluoride) or a functionality that can be activated within the active site through protonation by an enzyme amino acid residue that acts as a general acid (e.g. OH or OR, where R may be a simple alkyl or aromatic substituent or even another carbohydrate). For Gly-Ts the leaving groups are either nucleotide phosphate (mono- or di-) esters or just phosphate groups.

(ii) *Regioselectivity*: The enzyme effectively determines which groups will react through the precise orientation of reactants into their corresponding binding sites. The level of control typically depends on the enzyme class (e.g. Gly-Ts tend to have a high specificity for both the donor sugar and acceptor, yet glycosidases are often only specific for donor and may be very tolerant of a wide range of acceptors).

(iii) *Stereoselectivity*: As for regioselectivity, the shape of the enzyme active site and the mechanism involved typically results in very high stereoselectivities indeed. Both classes of enzyme may be further subdivided according to the change of anomeric configuration from product to substrate (*inverting* or *retaining*), and also according to the absolute configuration of the bond type that they process. Thus, an inverting  $\beta$ -galactosyltransferase creates a  $\beta$ -bonded galactoside using an  $\alpha$ -galactosyl nucleotidediphosphate donor and a retaining  $\beta$ -galactoside, cleaves  $\beta$ -galactoside bonds to give  $\beta$ -galactopyranose (and through transglycosylation may be used to create other  $\beta$ -galactosides).

Enzymes are often only treated as biological reagents but they are really no different to any other catalysts used in chemistry. Some general, key advantages of the use of enzymes in synthesis include specificity; little or no need for protecting groups; efficiency; activity under mild conditions; and they are environmentally benign (non-toxic and biodegradable). Certain disadvantages should also be acknowledged including over-specificity (it may be hard to use them as catalysts for a range of reactions, although rapid progress is being made in engineering broad

specificity catalysts); the occasional need for careful conditions (in some cases precise conditions of pH, cofactors and temperature may be needed); and sometimes limited availabilities of enzymes for key transformations (although the identification of putative function through the use of various genomes has greatly accelerated the isolation of novel activities).

Let us consider the practical use of the two types of carbohydrate processing enzymes in more detail.

## 12.2 GLYCOSIDASES

In order to avoid multi-step chemical syntheses to achieve regio- and stereospecific reactions, glycosidases have been employed as catalysts for glycoconjugate synthesis. Glycosidases are attractive for large-scale application since they are abundant catalysts, inexpensive, commercially available, and exhibit broad processing specificity, particularly with respect to the acceptor, as compared with more substrate specific glycosyltransferases [1].

The biological function of glycosidases is the cleavage of glycosidic linkages. There are two distinct classes of glycosidase enzymes: those hydrolysing the glycosidic bond by direct displacement of the leaving group with water, resulting in anomeric inversion, and those effecting transformation by a double displacement mechanism via a glycosyl-enzyme intermediate, effecting net retention [2]. Point-mutagenesis knockout experiments [3, 4] and inhibitor studies [5] with retaining glycosidases have shown that there are two acidic amino acid side chains (either glutamate or aspartate) that are separated by 5.5 Å within the active site of these enzymes. One of these catalytic residues acts as a nucleophile, attacking the anomeric carbon, which passes through an oxacarbenium ion-like transition state to form a glycosyl-enzyme intermediate. The other acid side chain acts as a general acid/base, protonating the hydroxyl leaving group and removing a proton from the water molecule acceptor. Figure 12.1 shows the catalytic mechanism of retaining glycosidases ( $R' = H$  and  $R = \text{sugar}$ ).

Under controlled conditions it is possible for glycosidases to catalyse the synthesis of glycosidic bonds instead of hydrolysis. Two methodologies are employed in such enzymatic glycosylations, namely thermodynamically controlled reverse hydrolysis and kinetically controlled transglycosylation.

Reverse hydrolysis involves a reaction between a monosaccharide donor with an acceptor, such as an alcohol or sugar, to give a glycoside and water (Figure 12.2). A high concentration of reactant carbohydrate is commonly used to force the equilibrium towards the glycoside product but this also enhances the unwanted formation of oligomeric side products. Lowering the thermodynamic activity of water ( $a_w$ ) in the reaction also enhances the yield of glycoside formation and so high temperatures and the use of organic cosolvents are also used. The approach is partially limited by the stability and activity of glycosidases under these conditions. Consequently, the yields for reverse hydrolysis reactions are rarely above 15%.

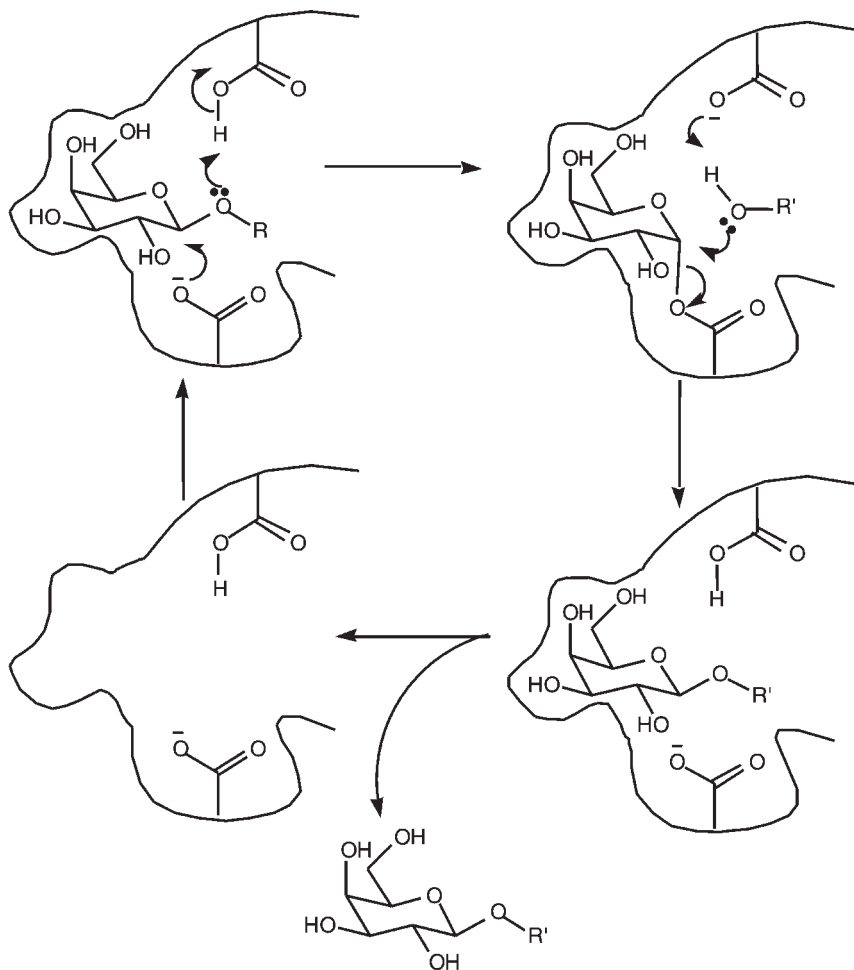


Figure 12.1

However, the starting materials are cheap and are often recycled after the reaction, and the reaction set-up is straightforward.

Transglycosylation utilises a reactive glycosyl donor in order to maintain a steady state concentration of glycosyl-enzyme intermediate. Ideally, this activated intermediate is rapidly intercepted by an excess of acceptor bound in the aglycon site, rather than water. The mechanism for retaining glycosidases with a *p*-nitrophenyl donor is shown in Figure 12.1 ( $R'$  = glycosyl acceptor and  $R$  = *p*-nitrophenyl). There is a dearth of literature evaluating the use of inverting glycosidases in synthesis; this is probably a consequence of their rarity.

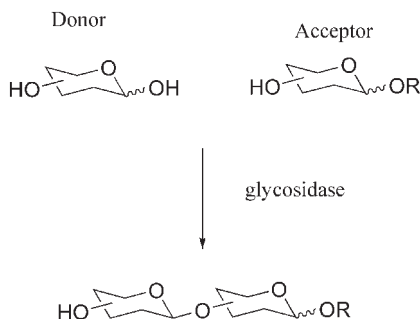


Figure 12.2

### 12.2.1 Preparation of activated donors

The activated donors in transglycosylation reactions fulfil three roles [6]:

- If the substrates of a reaction are more reactive than the products, the transglycosylation product will be favoured.
- Faster reacting donors keep reaction times short, reducing the time for possible product hydrolysis.
- Tightly binding (low  $K_M$ ) donors prevent or reduce product inhibition.

Several donors have been used for establishing kinetic control. By far the most prevalent in the literature are the *p*-nitrophenyl glycosides. They are efficiently recognised by glycosidases and their high reactivity enables ready glycosyl transfer within short timescales, thereby suppressing hydrolysis of products. However, their solubilities in water are low because of the hydrophobic *p*-nitrophenyl group. High concentrations of substrate aid formation of glycosylated products and therefore reactions are often carried out in organic enriched media if this is compatible with the enzyme in use.

*p*-Nitrophenyl- $\alpha$ -L-fuco/galacto/rhamno/manno-pyranosides can be made by the reaction of the acetate protected sugar with *p*-nitrophenol and zinc chloride at 120 °C under vacuum [7]. *p*-Nitrophenyl- $\beta$ -D-gluco/galactopyranoside can be generated from the reaction of the acetylated hexopyranosyl bromides with sodium *p*-nitrophenoxide in DMF [8], followed by deprotection, and *p*-nitrophenyl- $\beta$ -D-mannopyranoside can be synthesised from mannose via 2,3:4,6-di-*O*-cyclohexylidene- $\alpha$ -D-mannopyranose [9].

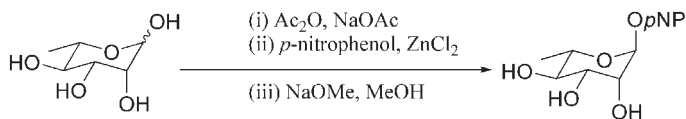
Galactosyl fluoride donors are reported to be more soluble in aqueous media than the corresponding nitrophenyl compound [10].  $\beta$ -D-Galactopyranosyl fluoride can be prepared by reaction of acetylated galactopyranosyl bromide with silver fluoride [10] or by reaction of penta-*O*-acetyl- $\beta$ -D-galactopyranoside with hydrogen fluoride in pyridine [11].

Disaccharides, such as lactose [12, 13], have also been used as donors in which the reducing end sugar acts as the leaving group.

### 12.2.2 Experimental procedure for preparation of activated donors

#### Method 1

The synthesis of *para*-nitrophenyl  $\alpha$ -L-rhamnoside [7] (Figure 12.3).



**Figure 12.3**

*Notes and discussion.* This pragmatic method allows preparation of *p*NP glycosides on a gram scale. In our hands, the material obtained after freeze drying is typically analytically pure and in these cases the final recrystallization may be omitted.

#### Materials.

L-Rhamnopyranose monohydrate (11.10 g, 60.9 mmol)	no known risk
Sodium acetate (5.5 g, 67.0 mmol)	irritant
Acetic anhydride (90 ml)	flammable, corrosive, reacts violently with water, lachrymator, toxic
Ice water (500 ml)	no risk
Chloroform (3 × 100 ml and 2 × 200 ml)	harmful, irritant, irreversible effects
Calcium chloride (drying agent)	irritant, harmful, avoid inhalation
<i>para</i> -Nitrophenol (25 g, 179.6 mmol)	irritant
Zinc chloride (6.88 g, 50.5 mmol)	corrosive, harmful
Solution of sodium hydroxide (1 M, 3 × 200 ml)	corrosive, harmful
Water (300 ml + 100 ml)	no risk
Magnesium sulfate (drying agent)	irritant, harmful, avoid inhalation
Methanol	flammable, toxic
Sodium methoxide solution in methanol (15 ml, 0.1 M)	flammable, toxic
Diethyl ether (2 × 100 ml)	extremely flammable, harmful
Ethanol	flammable, toxic

*Equipment.*

250 ml Round bottom flask  
 Reflux condenser  
 Heater stirrer with thermostat thermometer  
 Oil bath  
 Rotary evaporator  
 Water aspirator  
 1000 ml round bottom flask  
 Freeze dryer

*Special precautions.* Care should be taken with the extraction of the *p*NP-glycoside melt with chloroform: a temperature of 50 °C proves ideal for keeping the melt liquid but avoids overly rapid evaporation of the chloroform being used for the extraction.

*Procedure.* Stir the L-rhamnopyranose monohydrate (11.10 g, 60.9 mmol), sodium acetate (5.5 g, 67.0 mmol) and acetic anhydride (90 ml) in a 250 ml round bottomed flask fitted with a reflux condenser under an inert atmosphere. Heat the resulting mixture using an oil bath with a thermostat set to 110 °C for 90 min. Remove from the heat, and when cool pour over ice water (500 ml). After allowing to stand for 2 h, extract with chloroform (3 × 100 ml) and dry overnight (calcium chloride). After filtration, remove all the solvent *in vacuo* to yield a pale yellow oil. Combine this oil with *para*-nitrophenol (25 g, 179.6 mmol) in a 250 ml round bottom flask and heat at 120 °C, under the vacuum provided by a water aspirator, until all bubbling subsides. Add dry, finely powdered zinc chloride (6.88 g, 50.5 mmol) to the resulting melt and reapply the vacuum. Heat for 60 min at 120 °C during which time the mixture turns from pale yellow to light brown and then remove the vacuum and cool to 50 °C. At 50 °C extract with chloroform (200 ml × 2) and wash the resulting organic extract with a solution of sodium hydroxide (1 M, 3 × 200 ml) and then water (5 × 200 ml). Dry over magnesium sulfate, filter and remove the solvent *in vacuo*. Dissolve the resultant brown gum in refluxing methanol (100 ml), pass through a short, prewashed, activated charcoal column (5 × 5 cm), filter, and leave to stand overnight. Recrystallize the resulting pale brown crystals from hot methanol.  $[\alpha]_D^{22} = -121$  (CHCl<sub>3</sub>, *c* = 1.0); <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>) δ = 1.21 (d, *J*<sub>5,6</sub> = 6.2 Hz, 3H, H-6), 2.04, 2.06, 2.21 (s × 3, 3H × 3, -C(O)-CH<sub>3</sub> × 3), 3.90 (m, 1H, H-5), 5.17 (t, *J*<sub>3,4</sub> = 9.8 Hz, 1H, H-4), 5.45 (dd, *J*<sub>1,2</sub> = 1.6 Hz, *J*<sub>2,3</sub> = 3.5 Hz, 1H, H-2), 5.51 (dd, *J*<sub>2,3</sub> = 3.5 Hz, *J*<sub>3,4</sub> = 9.8 Hz, 1H, H-3), 5.56 (d, *J*<sub>1,2</sub> = 1.6 Hz, 1H, H-1), 7.19 (d, *J* = 7.1 Hz, 2H, Ar-H), 8.22 (d, *J* = 7.1 Hz, 2H, Ar-H); (dq, H × 1, H-5), 5.2–5.6. Dissolve the recrystallized material in dry methanol (250 ml) in a 1000 ml round bottom flask, and add a freshly prepared solution of sodium methoxide in methanol (15 ml, 0.1 M). Heat at reflux for 15 min and then remove the solvent. Dissolve the solid formed in water (100 ml), wash with diethyl ether (2 × 100 ml) to remove *p*-nitrophenol. Freeze dry the resulting aqueous layer and recrystallise from hot ethanol. Mp 171.4–173.0 °C;  $[\alpha]_D^{24} = -164.9$  (*c* 0.19, MeOH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ = 1.23 (d, *J*<sub>5,6</sub> = 6.06 Hz, 3H, H-6), 3.45 (t, *J*<sub>3,4</sub> = 9.35 Hz, 1H, H-4), 3.52 (dq, *J*<sub>5,6</sub> = 6.06 Hz, *J*<sub>4,5</sub> = 9.35 Hz, 1H, H-5), 3.81

(dd,  $J_{2,3} = 3.28$  Hz,  $J_{3,4} = 9.35$  Hz, 1H, H-3), 4.00 (dd,  $J_{1,2} = 1.77$  Hz,  $J_{2,3} = 3.28$  Hz, 1H, H-2), 5.61 (d,  $J_{1,2} = 1.77$  Hz, 1H, H-1), 7.22 (d,  $J = 9.35$  Hz, 2H, arH  $\times$  2), 8.22 (d,  $J = 9.35$  Hz, 2H, Ar-H  $\times$  2); Found: C, 50.22%; H, 5.52%; N, 5.31%; calcd: C, 50.53%; H, 5.30%; N, 4.91%.

### 12.2.3 Temperature and use of organic solvent

The temperature employed for enzyme mediated reactions will often be a compromise between the optimal temperature of enzyme activity, and the advantages of high temperature. At elevated temperatures lipophilic donors such as *p*-nitrophenyl glycosides are more soluble, enhancing yields of transglycosylation reactions. Similarly, these glycosyl donors are also more soluble in organic solvents. In the case of reverse hydrolysis, high temperature and organic solvents function to reduce the water activity of the reaction mixture, decreasing the rate of product hydrolysis.

The amount and type of cosolvent tolerated by glycosidases from different sources is extremely variable. For example, almond  $\beta$ -glucosidase requires an  $a_w$  of 0.4–0.5 for most alcoholic solvents, and 0.8 for acetonitrile, octanol and *tert*-butyl alcohol and was stable at 40 °C in 90% aqueous acetonitrile (CH<sub>3</sub> CN:buffer, 9:1 v/v) for several days, whereas in 30% aqueous acetonitrile it was rapidly deactivated [6]. This is probably a consequence of polar organic solvents causing structural changes at lower concentrations, but at high concentrations the enzyme is frozen in the active conformation due to the loss of water. Therefore, it is suggested that a screen of cosolvents and cosolvent/buffer ratios is carried out for new reactions to optimise yields. Thermophilic glycosidases are of great synthetic use as they are generally more tolerant of organic solvent than their mesophilic counterparts and can, by definition, be used at higher temperatures [6, 14].

Methods for maintaining glycosidase catalytic activity in non-aqueous media would greatly improve their synthetic utility. One possible system is the coating of glycosidases with a lipid monolayer, resulting in an enzyme preparation that is stabilised from deactivation and also soluble in most organic solvents [15, 16]. Using this method a  $\beta$ -galactosidase was solubilised in isopropyl ether and transglycosylation activity was observed.

### 12.2.4 Solvent volume

Product inhibition by *p*-nitrophenol has been reported in transglycosylation reactions [17, 18]. This was minimised by diluting *p*-nitrophenol concentration by using larger volumes of solvent in reactions.

### 12.2.5 Product trapping

Product trapping to minimise hydrolysis of the glycoside product is an alternative to using high temperature and organic solvent. For example, the hydrolysis by a sialidase

[19] or  $\beta$ -galactosidase [20] of transglycosylation products has been blocked by glycosyltransferase-mediated conversion into further oligosaccharides, which are poor substrates for the glycosidase. In this way, one-pot syntheses containing two enzymes catalyse the irreversible formation of products with improved yields.

By the sequential use of galactose oxidase and  $\beta$ -galactosidase, *N*-acetyl-D-lactosamine derivatives were synthesised in 60% yields [21]. The  $\beta$ -galactosidase will accept 6-oxo-D-galactose, prepared by the oxidation of the primary hydroxyl of galactose by galactose oxidase, as a glycosyl donor. Furthermore, the transglycosylation of this galactose derivative is favoured over glycoside hydrolysis. The desired product could be acquired by sodium borohydride reduction of this product.

### 12.2.6 Optimisation of time

One difficulty associated with transglycosylation reactions is that the product glycoside is also a substrate for glycosidase mediated hydrolysis. Following the reaction profile, the glycosyl donor is consumed and the concentration of the product increases. Product concentration will peak when the rates of synthesis and hydrolysis become equal; at this point kinetic control is lost and the reaction should be stopped before thermodynamic control proceeds and enzymatic hydrolysis consumes the product. Various methods have been employed to monitor reaction progress such as HPLC, thin layer chromatography (TLC) and UV absorption, following the release of *p*-nitrophenol at 405 nm. Optimisation of the timescale for each reaction is essential for attaining the best yields. It should be noted that, of course, this problem is avoided for glycosynthases (*vide infra*), which have no hydrolytic activity.

### 12.2.7 Regioselectivities

The regioselectivities of reactions mediated by glycosidases are assorted and variable. A sample of results is shown in Table 12.1. It has been suggested for increasing acceptor bulk (monosaccharide  $\rightarrow$  disaccharide  $\rightarrow$  trisaccharide, etc.),

**Table 12.1**

Examples of the synthetic regioselectivities of glycosidases

Enzyme	Source	Regioselectivity	References
$\beta$ -D-galactosidase	Bovine testes	1 $\rightarrow$ 3	29, 98
$\beta$ -D-galactosidase	<i>E. coli</i>	1 $\rightarrow$ 4 (1 $\rightarrow$ 3), 1 $\rightarrow$ 6	10, 29
$\beta$ -D-galactosidase	<i>B. circulans</i>	1 $\rightarrow$ 4	22
$\beta$ -N-acetylhexosaminidase	<i>A. oryzae</i>	1 $\rightarrow$ 4 (1 $\rightarrow$ 6), 1 $\rightarrow$ 6	22, 23, 29, 30
$\alpha$ -L-fucosidase	<i>P. multicolor</i>	1 $\rightarrow$ 3	24
$\alpha$ -L-fucosidase	Bovine kidney	1 $\rightarrow$ 4	98



that the binding conformation at the aglycon site increases the selectivity of transfer, resulting in a single predominating product [22].

In entry 4, the major product selectivity changes from  $1 \rightarrow 4$  to  $1 \rightarrow 6$  upon varying the acceptor from glucosamine [23] to galactosamine [22], with *p*-nitrophenyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside/galactopyranoside donors. This exemplifies how simple changes in the reactants can have unexpected effects on the products formed.

It must be noted that the example in entry 3 highlights an enzyme that adds a glycosyl unit regiospecifically at the  $1 \rightarrow 4$  position, notoriously the least reactive position in glycosyl acceptors [22].

The  $\alpha$ -fucosidase from *Penicillium multicolor* hydrolyses  $1 \rightarrow 2$ ,  $1 \rightarrow 3$  and  $1 \rightarrow 6$  disaccharide substrates. However, this is incompatible with data illustrating that only the  $1 \rightarrow 3$  regioisomer is made by transglycosylation. An explanation for this behaviour is that the glycosyl donor is hydrolysed at a similar rate to the  $1 \rightarrow 6$  and  $1 \rightarrow 2$  adducts, but the  $1 \rightarrow 3$  disaccharide is hydrolysed much more slowly. Therefore, these products are hydrolysed as soon as they are formed and the minor  $1 \rightarrow 6$  hydrolysis substrate is accumulated in the reaction mixture [24].

This note of caution illustrates that it is important to empirically determine whether the regioselectivities observed in glycosidase-catalyzed synthesis will bear any resemblance to those found in the corresponding hydrolytic reactions.

### 12.2.8 Purification

Even in the ideal situation, where the enzyme of choice is exceptionally regioselective and no oligomeric products are formed, a method of purifying product from excess starting materials and byproducts such as *p*-nitrophenol (from activated glycosyl donors) is needed. Some products may be crystalline but in most cases a more complex method for isolating the desired product from multiple byproducts is required. There are numerous methods [25] including preparative HPLC [10, 26], ion exchange chromatography [19, 27], gel-filtration chromatography [28], flash chromatography [21, 29] and carbon–Celite<sup>®</sup> chromatography [30]. The use of these is highlighted in the glycosidase-mediated syntheses described below.

### 12.2.9 Recycling the enzyme

It should be briefly mentioned that glycosidases have also been used whilst immobilised on a powdered nylon column [11] and a non-ionic Amberlite resin [31] so that the enzyme can be recycled for several experiments.

### 12.2.10 General notes on the practical use of glycosidases

Many of the methods used to illustrate the diverse reaction conditions employed for glycosidase mediated oligosaccharide synthesis have little or incomplete characterisation. Yields are calculated in a range of ways; from the limiting reagent

or the percentage of the crude product mixture that is the desired compound. Thus an unfortunate, general observation from the literature in this area is that preparative yields may differ quite markedly from those quoted.

Another problem is the units used for detailing the amount of enzyme used for these biotransformations. Quantities are often given in units (U), but this is rarely accompanied by a definition of what a unit represents and this value changes between different enzymes, commercial sources and grades of purification. This is an issue that may obscure the use of glycosidases in the synthetic laboratory but the uninitiated should not be discouraged.

### 12.2.11 Examples of thermodynamically controlled reverse hydrolysis reactions

#### Method 2

The synthesis of 2-pentyl glucoside using a thermophilic  $\beta$ -glycosidase from *Sulfolobus solfataricus* in organic solvent [14] (Figure 12.4).

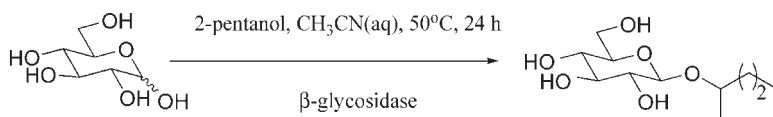


Figure 12.4

*Notes and discussion.* Alkyl glycoside formation has been used as a model system for monitoring the effects of reaction conditions on reverse hydrolysis, without the added complication of obtaining regioisomeric products. This synthesis uses a thermophilic enzyme in acetonitrile. The alcohol acceptor is used in a five-fold excess. The experiment also investigated the effect of different solvents and substrates on the enantioselectivity of acceptor addition. In spite of the utilisation of high temperature and organic solvent to minimise glycoside hydrolysis, the resulting yield is low.

#### Materials.

D-(+)-Glucose (0.36 g, 2 mmol)	no reported risks
2-Pentanol (1.1 ml, 10 mmol)	flammable, harmful
$\beta$ -Glycosidase from <i>S. solfataricus</i> (1 mg)	no reported risks
Acetonitrile (4.5 ml)	flammable, toxic
Distilled water (500 $\mu$ l)	no reported risks
Phosphate buffer (10 mM, pH 6.5)	no reported risks
Chloroform for chromatography	harmful, irritant, irreversible effects
Methanol for chromatography	flammable, toxic
Water for chromatography	no reported risks

*Equipment.*

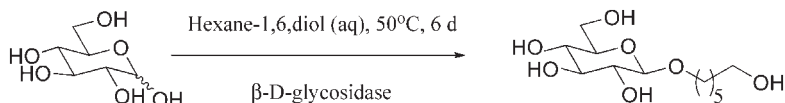
Sealed (10 ml) reaction vessel  
 Syringes (5 and 2 ml) and needles  
 Gilson pipette (1000 ml) and tips  
 Water bath and thermometer  
 TLC equipment  
 Rotary evaporator  
 Freeze dryer  
 Flash chromatography equipment

*Special precautions.* There are no reported risks associated with the glycosidase; however, many enzymes are immunogenic, so skin contact and inhalation of enzyme powders should be avoided. Safety glasses and gloves should be worn during this experiment and work should be carried out in a fume hood.

*Procedure.* Preincubate D-(+)-glucose (0.36 g, 2 mmol) and 2-pentanol (1.1 ml, 10 mmol) in distilled water (500  $\mu$ l) and acetonitrile (4.5 ml) at 50 °C. Add 10  $\mu$ l of enzyme solution containing 1 mg of  $\beta$ -D-glycosidase from *S. solfataricus* dissolved in phosphate buffer (10 mM, pH 6.5). Product formation can be monitored by TLC by removing 1–5  $\mu$ l aliquots at intervals and staining plates by spraying with orcinol–ferric chloride and developing at 100 °C. The reaction should be terminated after 24 h, when least free glucose remains, by initiating product purification. Concentrate the reaction mixture by rotary evaporation and then lyophilise. Dissolve this preparation in methanol (400  $\mu$ l) and separate by flash chromatography using chloroform/methanol/water (25:10:1, v/v). Fractions containing reaction products should be pooled, solvent removed by rotary evaporation and lyophilised to yield 54:46 R:S glucosides. The exact yield of glycoside in the procedure was not determined and the ratios of anomeric configurations were calculated by  $^1\text{H}$  NMR comparison of the racemic products with authentic chiral preparations. No characterisation was given. In the authors' laboratory, yields of 7–15% are typical for such a preparation.

*Method 3*

The synthesis of 6'-hydroxyhexyl  $\beta$ -D-glucopyranoside using almond  $\beta$ -D-glycosidase (Figure 12.5).



**Figure 12.5**

*Notes and discussion.* This synthesis is performed at high temperature and the alcohol glycosyl acceptor is used as the solvent (and therefore in huge excess) in order to increase the yield of alkyl glycoside to 60%.

*Materials.*

Glucose (0.9 g, 5 mmol)	no reported risks
Hexane-1,6-diol (22.5 ml)	flammable, harmful
Almond $\beta$ -glucosidase (125 mg)	no reported risks
Distilled water (2.5 ml)	no reported risks
Phosphate buffer (10 mM, pH 6.5)	no reported risks
Ethyl acetate	flammable, irritant, avoid inhalation/skin contact
Dichloromethane for chromatography	irreversible effects
Methanol for chromatography	flammable, toxic
Water for chromatography	no reported risks

*Equipment.*

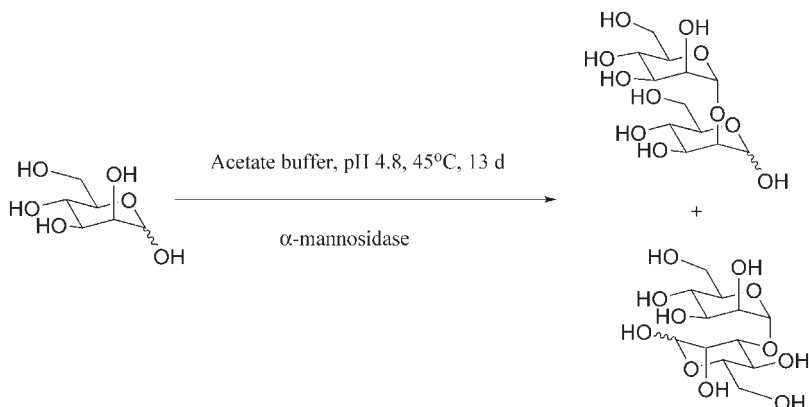
Sealed reaction vessel (50 ml)  
 Measuring cylinder  
 Syringe (5 ml) and needle  
 Shaking incubator or water bath  
 Filtration equipment  
 Separating funnel  
 Rotary evaporator  
 Flash chromatography equipment

*Special precautions.* As in Method 2.

*Procedure.* Dissolve the glucose (0.9 g, 5 mmol) in distilled water (2.5 ml) and hexane-1,6-diol (22.5 ml) in the sealed flask. Add almond  $\beta$ -D-glucosidase as a solution in a small amount of buffer and incubate at 50 °C with shaking at 110 rpm. After incubation for six days, remove the enzyme by filtration and extract the excess alcohol by separation between ethyl acetate and water. Concentrate the aqueous layer under vacuum and purify the product by flash chromatography using dichloromethane/methanol/water (40:10:1). This will yield 6'-hydroxyhexyl  $\beta$ -D-glucopyranoside (0.85 g, 61%) as an oil.  $[\alpha]_D^{27} = -31.6$  (c 0.28, H<sub>2</sub>O); <sup>1</sup>H NMR (D<sub>2</sub>O, selected data) 1.21–1.31 (m, O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>–, 4H), 1.50–1.64 (m, OCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub> 4H), 3.21 (dd, *J* = 8.0, 9.3 Hz, H-2, 1H), 3.31–3.47 (m, OCH<sub>2</sub>–, 1H), 3.68 (dd, *J* = 12.3, 5.8 Hz, H-6, 1H), 3.89 (m, H-6', OCH<sub>2</sub>, 2H), 4.41 (d, *J* = 7.9 Hz, H-1, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O) 25.4 (O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>–), 29.3 (OCH<sub>2</sub>CH<sub>2</sub>–), 31.8 (O(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>–), 61.4 (C-6), 62.4 (O(CH<sub>2</sub>)<sub>5</sub>CH<sub>2</sub>–), 70.3, 71.2, 73.8, 76.5 (C-2, 3, 4, 5), 102.8 (C-1); HRMS *m/z* 303.1415 (M + Na)<sup>+</sup> C<sub>12</sub>H<sub>24</sub>O<sub>7</sub> requires 303.1419.

*Method 4*

The synthesis of 2-*O*-(D-mannopyranosyl)-D-mannopyranose and 3-*O*-(D-mannopyranosyl)-D-mannopyranose using  $\alpha$ -mannosidase from almond meal [32] (Figure 12.6).



**Figure 12.6**

*Notes and discussion.* This reaction illustrates the formation of a disaccharide by reverse hydrolysis; the reaction time of 13 days is typical in spite of the high temperature and high substrate concentration.

*Materials.*

Almonds (95 g)	no reported risks
Acetone for washing	flammable, irritant
Hexane for washing	flammable, irritant, impairs fertility
Citrate–phosphate buffer (50 mM, pH 5.0)	no reported risks
Sodium acetate buffer (20 mM, pH 4.5)	no reported risks
Mannose (4 g, 22 mmol)	no reported risks
Distilled water (2.5 ml)	no reported risks
Sodium acetate buffer (1.25 ml, 0.1 M, pH 4.8)	no reported risks
Ethanol for chromatography	flammable
Water for chromatography	no reported risks

*Equipment.*

Blender  
Filtration apparatus  
Separating funnel  
Ammonium sulfate precipitation equipment  
Gel filtration chromatography equipment  
Round bottomed flask (10 ml) with rubber septum  
Syringes (2 × 2 ml) and needles  
Water bath and thermometer  
Rotary evaporator  
Carbon–Celite<sup>®</sup> chromatography equipment

*Special precautions.* There are no reported risks associated with the glycosidase; however, many enzymes are immunogenic, so skin contact and inhalation of enzyme powders should be avoided. Safety glasses and gloves should be worn during this experiment and work should be carried out in a fume hood.

*Procedure.* Almonds (95 g) should be washed in distilled water, rinsed with acetone and blotted dry. Homogenise the almonds in a blender and add acetone (200 ml) over dry ice. The finely ground material should be filtered through a fine cloth and washed with chilled acetone followed by hexane. Residual solvent should be removed by rotary evaporation to give dry almond meal (39 mg). Almond meal (10 g) should be extracted with sodium acetate buffer (20 mM, pH 4.5) for 30 min, filtered and centrifuged. The proteins can be fractionated by ammonium sulfate precipitation (35–75% saturation) and the precipitate can be collected by centrifugation and dialysed against the same buffer. The dialyte should be purified by gel-filtration (CM-Sephrose, 13 × 2.6) and eluted with an increasing salt concentration (0–0.4 M NaCl) in the same buffer.  $\alpha$ -Mannosidase activity of the eluted fractions is measured and the fractions containing activity should be pooled. The activity of the  $\alpha$ -mannosidase can be determined by measuring *p*-nitrophenol absorbance at 400 nm released from the incubation of enzyme with *p*-nitrophenyl  $\alpha$ -D-mannopyranoside (5 mM) in citrate–phosphate buffer (50 mM, pH 5.0) at 30 °C for 10 min. 1 U is defined as the amount of enzyme releasing 1  $\mu$ mol of *p*-nitrophenol per minute from 5 mM *p*-nitrophenyl- $\alpha$ -D-mannopyranoside at pH 5.0 and 30 °C.

Incubate mannose (4 g, 22 mmol) in sodium acetate buffer (1.25 ml, 0.1 M, pH 4.8) with  $\alpha$ -mannosidase from almond meal (1 ml, 6.3 U ml<sup>-1</sup>) for 13 days at 45 °C. Heating the vessel in boiling water for 5 min stops the reaction by denaturing the enzyme. The reaction mixture can be purified by carbon–Celite<sup>®</sup> chromatography (290 g, 1:1), washing with water and then eluting with a 2% (4 l), 3% (1 l) and 4% (1 l) ethanol solution to give starting material and 2-*O*-(D-mannopyranosyl)-D-mannopyranose (190 mg, 5%) as a mixture of  $\alpha$ : $\beta$  = 88:12, and 3-*O*-( $\alpha$ -D-mannopyranosyl)-D-mannopyranose (113 mg, 3%) as a mixture of  $\alpha$ : $\beta$  = 70:30.

Disaccharide 2-*O*-(D-mannopyranosyl)-D-mannopyranose:  $[\alpha]_D^{22} = +38.7$  (*c* 1.19, H<sub>2</sub>O); <sup>1</sup>H NMR (D<sub>2</sub>O) 5.34 (d, 0.88H, *J* = 1.3 Hz, H-1), 5.10 (d, 0.12H, *J* = 1.6 Hz, H-1'), 4.99 (d, 0.88H, *J* = 1.6 Hz, H-1'), 4.87 (d, 0.12H, *J* = 1.3 Hz, H1) 4.07–4.05 (m, 0.12H, H-2'), 4.03 (dd, 1H, *J* = 3.2 and 1.6 Hz, H-2'), 3.91–3.56 (m, 10.88H), 3.33–3.39 (m, 0.12H); 5.34 (d, *J* = 1.32 Hz, H-1α, 0.88H), 5.10 (d, *J* = 1.64 Hz, H-1β, 0.12H), 4.99 (d, *J* = 1.6 Hz, H-1α, 0.88H), 4.87 (d, *J* = 1.3 Hz, H-1β, 0.12H), 4.07–4.05 (m, H-2', 0.12H), 4.03 (dd, *J* = 3.22, 1.6 Hz, H-2', 1H), 3.91–3.56 (m, 0.88H), 3.33–3.39 (m, 0.12H); <sup>13</sup>C NMR (D<sub>2</sub>O) 102.82, 102.07, 94.07, 93.16, 79.75, 78.81, 77.34, 74.05, 73.86, 73.48, 73.09, 70.94, 70.75, 70.60, 67.67, 67.49, 67.38, 67.27, 61.69, 61.58, 62.51; *m/z* 365.1060 (M + Na)<sup>+</sup>. C<sub>12</sub>H<sub>22</sub>NaO<sub>11</sub> requires 365.1054.

### 12.2.12 Examples of kinetically controlled transglycosylation reactions

#### Method 5

The synthesis of *p*-nitrophenyl 4-*O*-(β-D-galactopyranosyl)-β-D-xylopyranoside and *p*-nitrophenyl 3-*O*-(β-D-galactopyranosyl)-β-D-xylopyranoside using β-galactosidase from *Escherichia coli* and an activated fluoride donor [10] (Figure 12.7).

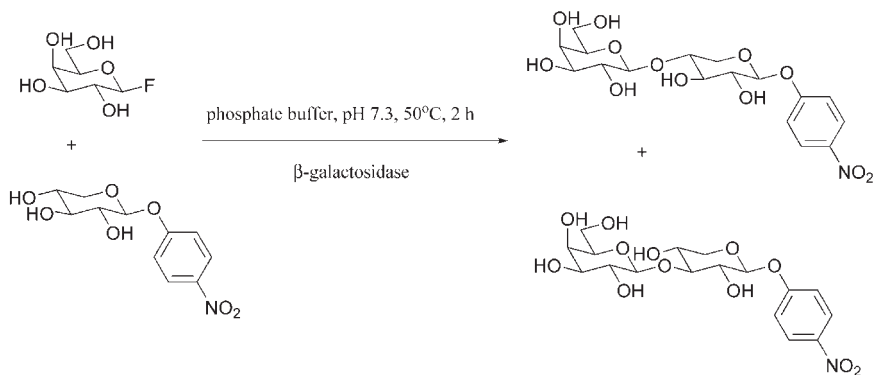


Figure 12.7

**Notes and discussion.** A fluoride glycosyl donor is used due to its high solubility in aqueous media. This means that higher concentrations of donor are available for the reaction (in comparison to a tandem reaction using a *p*-nitrophenyl donor), thereby increasing the transglycosylation yield [10]. A high concentration of phosphate buffer is required in the reaction to neutralise the hydrogen fluoride liberated during the reaction, and thus maintain the optimum pH for *E. coli* β-galactosidase. This synthesis confirms that the enzyme active site will accommodate modified sugar acceptors such as *p*-nitrophenyl β-D-xylopyranoside. The *p*-nitrophenyl group serves to protect the reducing end of the sugar and enables facile reversed phase HPLC owing to its strong UV absorption and appropriate lipophilicity.

*Materials.*

$\beta$ -D-Galactopyranosyl fluoride (141 mg, 0.774 mmol)	assume toxic
<i>p</i> -Nitrophenyl $\beta$ -D-xylopyranoside (70 mg, 0.258 mmol)	assume toxic
$\beta$ -Galactosidase from <i>E. coli</i> (Sigma Chemical Co.) (50 U) (1 U will hydrolyse 1.0 $\mu$ mol of <i>o</i> -nitrophenyl- $\beta$ -D-galactopyranoside to <i>o</i> -nitrophenol and galactose per minute at pH 7.3 and 37 °C)	no reported risk
Phosphate buffer (1.30 ml, 1.0 M, pH 7.3)	no reported risk
Acetonitrile for chromatography	flammable, toxic

*Equipment.*

Round bottomed flask (10 ml) with rubber septum  
 Syringe (2 ml) and needle  
 Water bath and thermometer  
 Filter paper and Buchner flask  
 Water pump  
 Rotary evaporator  
 Freeze dryer  
 HPLC (Shimadzu LC-6AD liquid chromatograph fitted with a YMC-GEL ODS-M 120 S-5 column, 20  $\times$  250 mm)

*Special precautions.* There are no reported risks associated with the  $\beta$ -galactosidase from *E. coli*, however, many enzymes are immunogenic, so avoid skin contact and inhalation of enzyme powders. Safety glasses and gloves should be worn during this experiment.

*Procedure.* Add  $\beta$ -galactosidase (50 U) to a solution of  $\beta$ -D-galactopyranosyl fluoride (141.0 mg, 0.774 mmol) and *p*-nitrophenyl  $\beta$ -D-xylopyranoside (70 mg, 0.258 mmol) in phosphate buffer (1.0 M, pH 7.3, 1.30 ml) at 50 °C. Allow the mixture to stand for 2 h before stopping the reaction by denaturing the enzyme at 80 °C for 1 min. Filter the mixture and concentrate the filtrate by rotary evaporation. This residue can be purified by HPLC using 18% H<sub>2</sub>O–CH<sub>3</sub>CN, with a flow rate of 8 ml min<sup>-1</sup> and by detecting product by UV absorbance at 320 nm. The products elute after 16.9 and 27.1 min and after lyophilisation, the reaction yields *p*-nitrophenyl-4-*O*-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-xylopyranoside (36.8 mg, 55.1%) and *p*-nitrophenyl-3-*O*-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-xylopyranoside (3.6 mg, 3.2%), respectively.

*p*-Nitrophenyl-4-*O*-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-xylopyranoside: [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -64.9 (c 4.95, H<sub>2</sub>O); <sup>1</sup>H NMR (D<sub>2</sub>O) 8.14 (d, *J* = 9.2 Hz, *p*NP, 2H), 7.12 (d, *J* = 9.4 Hz, *p*NP, 2H), 5.13 (d, *J* = 7.6 Hz, H-1, Xyl, 1H), 4.37 (d, *J* = 7.8 Hz, H-1, Gal, 1H), 4.07 (dd, *J* = 12, 5.2 Hz, H-5, Xyl, 1H), 3.84 (m, H-4, Xyl, 1H), 3.79 (d, *J* = 3.2 Hz, H-4, Gal, 1H), 3.64–3.55 (m, 6H), 3.51 (dd, *J* = 7.2, 11.0 Hz, H-2, Gal, 1H); <sup>13</sup>C NMR

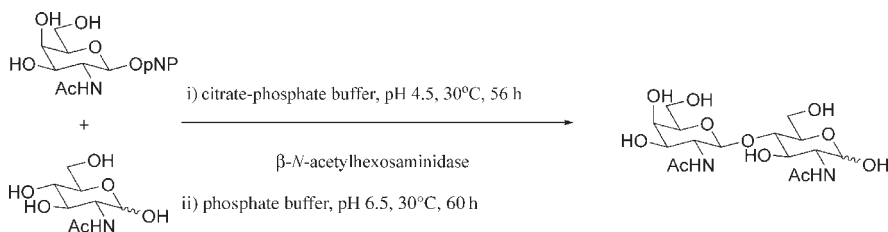


(D<sub>2</sub>O) 160.3 (*p*NP), 141.4 (*p*NP), 124.9 (*p*NP), 116.3 (C-2, *p*NP), 101.0 (C-1, Gal), 98.6 (C-1, Xyl), 75.0 (C-4, Xyl), 74.1, 72.4, 71.2, 69.4 (C-2, Gal), 67.4 (C-4, Gal), 62.0 (C-5, Xyl), 59.9 (C-6, Gal); FAB-MS *m/z* 434.2 (M + H)<sup>+</sup>. Found: C, 47.11; H, 5.31; N 3.29%. Calcd for C<sub>17</sub>H<sub>23</sub>NO<sub>12</sub>: C, 47.12, H, 5.35; N, 3.23%.

*p*-Nitrophenyl 3-*O*-(β-D-galactopyranosyl)-β-D-xylopyranoside: <sup>1</sup>H NMR (D<sub>2</sub>O) 7.71 (d, *J* = 8.8 Hz, *p*NP, 1H), 7.21 (d, *J* = 8.8 Hz, *p*NP, 2H), 5.21 (d, *J* = 7.4 Hz, H-1, Xyl, 1H), 4.33 (d, *J* = 7.7 Hz, H-1, Gal, 1H), 4.15 (dd, *J* = 4.9, 10.9 Hz, H-5eq, Xyl, 1H), 3.62 (d, *J* = 2.9 Hz, H-4, Gal, 1H), 3.80–3.73 (m, H-4, Xyl, 2H), 3.70 (dd, *J* = 3.4 Hz, H-3, Xyl, 1H), 3.61–3.55 (m, 6H); <sup>13</sup>C NMR (D<sub>2</sub>O) 162.4 (*p*NP), 143.6 (*p*NP), 127.0 (*p*NP), 117.4 (*p*NP), 104.1 (C-1, Gal), 100.7 (C-1, Xyl), 84.4 (C-3, Xyl), 76.3, 73.5, 73.1, 72.2, 69.5, 68.7, 65.8 (C-5, Xyl), 62.0 (C-6, Gal); FAB-MS *m/z* 434.2 (M + H)<sup>+</sup>.

### Method 6

The synthesis of 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-D-glucopyranose using β-*N*-acetylhexosaminidases from *Aspergillus oryzae* and Jack bean with a *p*-nitrophenyl activated donor [23] (Figure 12.8).



**Figure 12.8**

**Notes and discussion.** This method describes the use of β-*N*-acetylhexosaminidase from *A. oryzae*. A mixture of the β-(1 → 4) (major) and β-(1 → 6) (minor) adducts plus reverse hydrolysis products are produced. However, incubation with β-*N*-acetylhexosaminidase from Jack beans (*Canavalia ensiformis*) selectively hydrolyses the β-(1 → 6) product and the reverse hydrolysis products to yield the pure β-(1 → 4)-disaccharide. This method illustrates how the specificities and activities of different glycosidases can be combined to create the desired regioisomer. A full procedure for carbon–Celite<sup>®</sup> chromatography of monosaccharides from disaccharides and higher oligomers is given [30].

### Materials.

*p*-Nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside    assume toxic  
(0.4 g, 1.17 mmol)

*N*-Acetyl-D-glucosamine (2.56 g, 11.57 mmol)    no reported risks

$\beta$ - <i>N</i> -Acetylhexosaminidase from <i>A. oryzae</i> (Sigma Chemical Co.) (1 ml, 242.5 mg protein ml <sup>-1</sup> , 5.84 × 10 <sup>-3</sup> U mg <sup>-1</sup> protein)	no reported risks
Citrate-phosphate buffer (10 ml, 0.05 M, pH 4.5)	no reported risks
$\beta$ - <i>N</i> -Acetylhexosaminidase from Jack beans (Sigma Chemical Co.) (0.3 ml, 1 mg protein ml <sup>-1</sup> , 56 U mg <sup>-1</sup> protein)	no reported risks
Phosphate buffer (6 ml, 0.04 M, pH 6.5)	no reported risks
Ethanol for chromatography	toxic, flammable

### Equipment.

Round bottomed flask (50 ml) with rubber septum  
Syringes (1 and 10 ml) and needles  
Water bath  
HPLC equipment  
Carbon–Celite<sup>®</sup> chromatography equipment

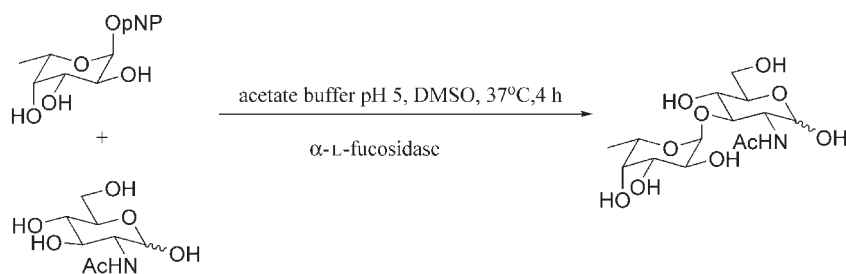
*Special precautions.* There are no reported risks associated with the enzymes used in this experiment; however, many enzymes are immunogenic, so avoid skin contact and inhalation of enzyme powders. Although the *p*-nitrophenyl donor has no reported risks, the by-product of the reaction, *p*-nitrophenol, is harmful if in contact with the skin, inhaled or swallowed and there is a risk of cumulative effects from repeated exposure. Safety glasses and gloves should be worn during this experiment.

*Procedure.* Suspend *p*-nitrophenyl 2-acetamido-2-deoxy- $\beta$ -D-galactopyranoside (0.4 g, 1.17 mmol) and *N*-acetyl-D-glucosamine (2.56 g, 11.57 mmol) in citrate–phosphate buffer (10 ml, 0.05 M, pH 4.5) in a round-bottomed flask. Heat this mixture at 45–50 °C for 2–3 min and 30 °C for 5 min. Add  $\beta$ -*N*-acetylhexosaminidase from *A. oryzae* (1 ml, 242.5 mg protein ml<sup>-1</sup>, 5.84 × 10<sup>-3</sup> U mg<sup>-1</sup> protein) and incubate at 30 °C for 56 h. HPLC can be used to determine the ratio of (1 → 4) to (1 → 6) ligation products. Heating the reaction vessel in a boiling water bath for 5 min stops the reaction. The mixture should be applied to a charcoal–Celite<sup>®</sup> column (45 cm × 3 cm) made up from activated carbon (Darco G-60, 100 mesh) and Celite<sup>®</sup> 535 (1:1, 70 g). The column is eluted with 5:95 ethanol/water to remove monosaccharide followed by 10:90 ethanol/water to recover the disaccharide mixture. The fractions containing the disaccharides should be evaporated to dryness under reduced pressure. Redissolve this residue in phosphate buffer (6 ml, 0.04 mM, pH 6.5) and incubate at 30 °C for 60 h with the  $\beta$ -*N*-acetylhexosaminidase from Jack bean (0.3 ml, 1 mg protein ml<sup>-1</sup>, 56 U mg<sup>-1</sup> protein). Stop the reaction by heating at 100 °C for 5 min and isolate the product disaccharide by carbon–Celite<sup>®</sup>

chromatography as described above to give 2-acetamido-4-*O*-(2-acetamido-2-deoxy- $\beta$ -D-galactopyranosyl)-2-deoxy-D-glucopyranose (0.352 g, 72%).  $[\alpha]_D^{25} = +42.3$  (c 0.52, H<sub>2</sub>O);  $^1\text{H}$  NMR (D<sub>2</sub>O) 1.97 (s, Me, 3H), 2.00 (s, Me, 3H), 3.44–3.90 (m, 12H), 4.46 (d,  $J = 8.40$  Hz, H-1',  $\beta$ ), 4.47 (d,  $J = 8.44$  Hz, H-1',  $\alpha$ ), 4.64 (d,  $J = 8.28$  Hz, H-1,  $\beta$ ), 5.13 (d,  $J = 2.92$  Hz, H-1,  $\alpha$ );  $^{13}\text{C}$  NMR (D<sub>2</sub>O) 22.55 (Me, reducing end  $\alpha$ ), 22.85 (Me, non-reducing end  $\beta$ ), 53.24 (C-2'), 54.22 (C-2,  $\alpha$ ), 56.64 (C-2,  $\beta$ ), 60.71 (C-6,  $\alpha$ ), 60.84 (C-6,  $\beta$ ), 61.62 (C-6'), 68.28 (C-4'), 70.00 (C-3,  $\alpha$ ), 70.64 (C-5,  $\alpha$ ), 71.36 (C-3'), 73.24 (C-3,  $\beta$ ), 75.25 (C-5,  $\beta$ ), 76.01 (C-5'), 79.72 (C-4,  $\beta$ ), 80.20 (C-4,  $\alpha$ ), 91.09 (C-1,  $\alpha$ ), 95.51 (C-1,  $\beta$ ), 102.40 (C-1'), 175.11 (C=O, reducing end  $\alpha$ ), 175.38 (C=O, reducing end  $\beta$ ), 175.43 (C=O, non-reducing end); FAB-MS  $m/z$  447.1601 ( $M + \text{Na}$ ); C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>11</sub> requires 447.1591.

### Method 7

The use of *P. multicolor*  $\alpha$ -L-fucosidase to synthesise 2-acetamido-3-*O*-( $\alpha$ -L-fucopyranosyl)-2-deoxy-D-glucopyranose using dimethylsulfoxide as cosolvent [24] (Figure 12.9).



**Figure 12.9**

**Notes and discussion.** This method illustrates the use of an organic solvent, DMSO, in transglycosylation reactions in order to enhance the solubility of the *p*-nitrophenyl donors, thus increasing the reaction yield. This example was used to compare the activity of  $\alpha$ -L-fucosidase from *P. multicolor* and *Aspergillus niger* in organic cosolvents and determined that the former was more active in higher organic cosolvent concentrations [24]. The enzyme was screened for activity in various concentrations and types of organic cosolvents and 10% DMSO offered a compromise between high enzyme activity and glycosyl donor solubility for this reaction. No characterisation was provided for the product and structure determination was made by comparing  $^{13}\text{C}$  NMR data to literature values.

### Materials.

<i>N</i> -Acetyl-D-glucosamine (500 mg, 2.3 mmol)	no reported risks
<i>p</i> -Nitrophenyl- $\alpha$ -L-fucopyranoside (100 mg, 0.35 mmol)	no reported risks
Lactase-P™ (K. I. Chemical Industry Co., Ltd, Shizuoka, Japan)	no reported risks

Dimethyl sulfoxide (DMSO) (2 ml)	irritant
Sodium phosphate buffer (10 l, 1 mM, pH 6.8)	no reported risks
Sodium phosphate buffer (5 l, 400 mM, pH 6.8)	no reported risks
Sodium acetate buffer (20 ml, 0.1 M, pH 5.0)	no reported risks
Methanol for carbon–Celite <sup>®</sup> chromatography	flammable, toxic

### *Equipment.*

Dialysis equipment—tubing, bucket, magnetic stirrer and bar  
Gel filtration chromatography equipment (Bio-Gel HTP, Biorad Labs, 2.6 × 11 cm)  
1.5 ml centrifuge tubes  
UV spectrophotometer set at 505 nm  
Ultrafree CL concentrator (Millipore, molecular cut off 30 kDa)  
Round bottomed flask (50 ml) with rubber septum  
Syringes (2 × 2 ml and 1 × 10 ml) and needles  
Water bath  
HPLC (AKTA-design system (Pharmacia) using a AccQ Tag column)  
TLC equipment and phenol–sulfuric acid stain  
Carbon–Celite<sup>®</sup> chromatography equipment  
Rotary evaporator

*Special precautions.* Remember that the source of the enzyme is bacterial and that some contamination may exist in the stock. Good microbiological technique should be practised. There are no reported risks associated with the  $\alpha$ -L-fucosidase from *P. multicolor*; however, many enzymes are immunogenic, so avoid skin contact and inhalation of enzyme powders. Although the *p*-nitrophenyl donor has no reported risks the by-product of the reaction, *p*-nitrophenol, is harmful if in contact with the skin, inhaled or swallowed and there is a risk of cumulative effects from repeated exposure. Reactions should therefore be carried out in a fume hood with eye protection and gloves.

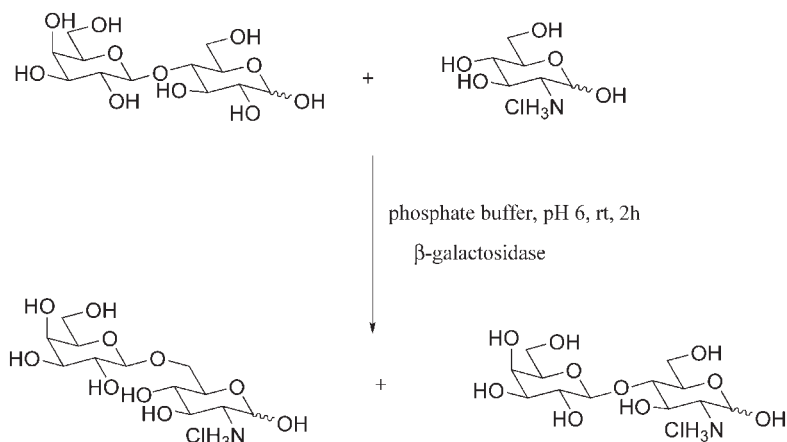
*Procedure.* The  $\alpha$ -L-fucosidase is prepared from Lactase-P<sup>™</sup>, a powdered culture broth of *P. multicolor* by gel filtration chromatography. Dissolve Lactase-P (1 g) in sodium phosphate buffer (10 ml, 1 mM, pH 6.8) and dialyse against the same buffer. Apply this dialyte to a gel-filtration column (Bio-Gel HTP, Biorad Labs, 2.6 × 11 cm) and elute with a gradient of 1–400 mM sodium phosphate buffer with a flow rate of 0.5 ml per minute. Collect 1 ml fractions and incubate 10  $\mu$ l of each fraction and 90  $\mu$ l of sodium acetate buffer (0.1 M, pH 5.0) with *p*-nitrophenyl- $\alpha$ -L-fucopyranoside (50  $\mu$ l, 5 mM) in a 1.5 ml centrifuge tube for 5 min at 37 °C. Fractions containing  $\alpha$ -L-fucosidase activity (yellow coloration due to release of *p*-nitrophenol) should be collected and concentrated to 0.5 ml using an Ultrafree CL (Millipore, molecular cut off 30 kDa). Enzyme activity can be determined by measuring the release of *p*-nitrophenol at 405 nm where 1 U corresponds to

the amount of enzyme that produces 1  $\mu\text{mol}$  of *p*-nitrophenol per minute from *p*-nitrophenyl- $\alpha$ -L-fucopyranoside. The activity of this enzyme solution should be  $\sim 4.4 \text{ U ml}^{-1}$ .

Add  $\alpha$ -L-fucosidase from *P. multicolor* (1.5 ml, 6.2 U) to a solution of *N*-acetyl-D-glucosamine (500 mg, 2.3 mmol) and *p*-nitrophenyl- $\alpha$ -fucopyranoside (100 mg, 0.35 mmol) in sodium acetate buffer (8.5 ml, 0.1 M, pH 5.0) containing DMSO (2 ml). This solution should be incubated at 37 °C and monitored by HPLC with a AccQ Tag column. When the peak of *p*-nitrophenyl- $\alpha$ -L-fucopyranoside disappears ( $\sim 4$  h) heat the vessel for 5 min in boiling water to stop the reaction. Isolate the product using carbon–Celite® chromatography (2.7  $\times$  50 cm) washing with 500 ml of water and eluting with a gradient of water (1 l)–30% methanol (1 l). TLC using phenol–sulfuric acid staining will detect the sugar containing fractions. Fractions containing disaccharide should be pooled and concentrated by rotary evaporation to give 2-acetamido-3-*O*-( $\alpha$ -L-fucopyranosyl)-2-deoxy-D-glucopyranose (63.5 mg, 49.3%). The product afforded  $^{13}\text{C}$  NMR chemical shifts (100.8, 101.0, 81.6, 79.3, 62.0, 61.9, 16.7, 16.3 ppm) which, by comparison with literature values suggested that the disaccharide was the desired product.

### Method 8

The use of  $\beta$ -galactosidase from *Bacillus circulans* to synthesise 4-*O*-( $\beta$ -D-galactopyranosyl)-2-amino-2-deoxy-D-glucopyranose and 6-*O*-( $\beta$ -D-galactopyranosyl)-2-amino-2-deoxy-D-glucopyranose [27] (Figure 12.10).



**Figure 12.10**

*Notes and discussion.* This reaction exemplifies the use of lactose as a glycosyl donor with the  $\beta$ -galactosidase from *B. circulans*. Lactose has previously been shown to give good regioselective glycosylation of various glycosides as acceptor

substrates [13]. A mixture of  $\beta$ -(1  $\rightarrow$  6) and  $\beta$ -(1  $\rightarrow$  4) regioisomers is produced but these can be separated using ion exchange chromatography by virtue of the enzyme compatible amino group present in the acceptor [27]. Incomplete characterisation is given for both compounds. This reaction is carried out in aqueous media and so the yield is limited due to competitive product hydrolysis.

*Materials.*

Lactose (6.84 g, 20 mmol)	no reported risk
Glucosamine hydrochloride (0.43 g, 2 mmol)	no reported risk
$\beta$ -Galactosidase from <i>B. circulans</i> (10 mg)	no reported risk
Phosphate buffer (10 ml, 50 mM, pH 6.0)	no reported risk
HCl for chromatography (0.05 M)	corrosive

*Equipment.*

Round bottomed flask (25 ml) with rubber septum and magnetic stirrer bar  
Magnetic stirrer  
Syringe (10 ml) and needle  
Freeze dryer  
Ion exchange chromatography equipment—cation exchange  
Filter paper, funnel and Buchner flask  
Water pump  
Anion exchange resin (Dowex, Marathon WBA, OH<sup>−</sup> form, 20–50 mesh)

*Special precautions.* Care should be taken when making phosphate buffer as the sodium phosphate salts are irritant to the eyes, skin and respiratory system. As in the transglycosylation methods, avoid contact or inhalation of the enzyme. Gloves and eye protection should be worn.

*Procedure.* Add lactose (6.84 g, 20 mmol), glucosamine hydrochloride (0.43 g, 2 mmol) and  $\beta$ -galactosidase from *B. circulans* (10 mg) to a round bottomed flask equipped with a magnetic stirrer and adjust the volume to 10 ml using phosphate buffer (50 mM, pH 6.0). The reaction should be left stirring for 2 h at room temperature. The reaction mixture should then be loaded onto a cation-exchange column. Unreacted starting materials and byproducts, which do not contain amino groups, will be removed with distilled water, and the column must be washed with a dilute HCl solution. The regioisomers 4-*O*-( $\beta$ -D-galactopyranosyl)-2-amino-2-deoxy-D-glucopyranose and 6-*O*-( $\beta$ -D-galactopyranosyl)-2-amino-2-deoxy-D-glucopyranose can be separated by elution with 0.05 M HCl. Monitor the elution by TLC analysis using a fluorescent indicator. The desired product fractions should be pooled and neutralized with an anion-exchange resin (Dowex, Marathon WBA, OH<sup>−</sup> form, 20–50 mesh) and concentrated under reduced pressure. Freeze drying yields the pure products as hydrochloride salts.

4-*O*-( $\beta$ -D-galactopyranosyl)-2-amino-2-deoxy-D-glucopyranose (0.75 g, 10%):  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , selected data) 5.31 (d,  $J = 3.6$  Hz, H-1,  $\alpha$ ), 4.85 (d,  $J = 8.4$  Hz, H-1,  $\beta$ ), 4.34 (d,  $J = 7.6$  Hz, H-1',  $\beta$ );  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , selected data) 103.30 (C-1',  $\beta$ ), 92.80 (C-1,  $\beta$ ), 89.11 (C-1,  $\alpha$ ); HRFABMS  $m/z$  342.1399 ( $\text{M}^+$ ).  $\text{C}_{12}\text{H}_{24}\text{NO}_{10}$  requires 342.1400.

6-*O*-( $\beta$ -D-galactopyranosyl)-2-amino-2-deoxy-D-glucopyranose (0.45 g, 6%):  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , selected data) 5.30 (d,  $J = 2.8$  Hz, H-1,  $\alpha$ ), 4.85 (d,  $J = 8.0$  Hz, H-1,  $\beta$ ), 4.36 (d,  $J = 7.2$  Hz, H-1',  $\beta$ );  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , selected data) 103.64 (C-1',  $\beta$ ), 93.11 (C-1,  $\beta$ ), 89.46 (C-1,  $\alpha$ ).

### Method 9

Synthesis of dimethylethylsilyl 3-*O*-(2- $\alpha$ -*N*-acetylneuraminy)- $\beta$ -D-galactopyranoside using a combination of *trans*-sialidase and sialyltransferase [28] (Figure 12.11).

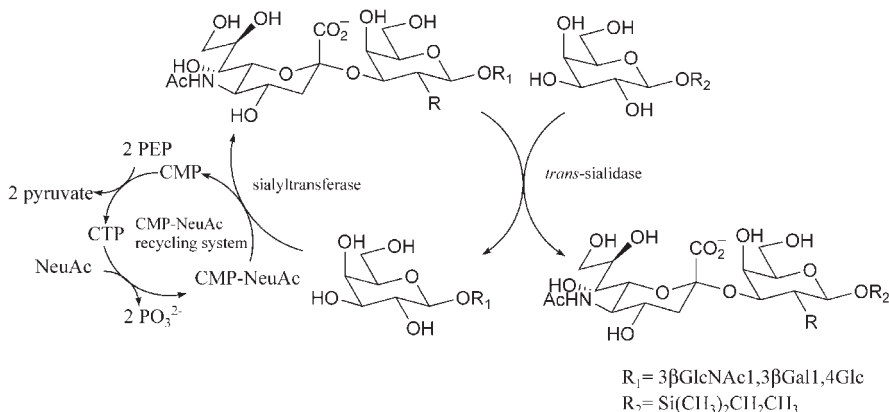


Figure 12.11

**Notes and discussion.** A major drawback of enzymatic sialylation with sialyltransferases is the strict acceptor specificity of these enzymes. This synthesis addresses this limitation by showing a widely applicable transfer of sialic acid (NeuAc) from a donor substrate of the sequence NeuAc $\alpha$ -(2 $\rightarrow$ 3)Gal-OR<sub>1</sub> to virtually any galactose acceptor (Gal-OR<sub>2</sub>). This method uses the less substrate specific glycosidase from *Trypanosoma cruzi*. The problem with this method is that the product is made at the expense of another sialoside, used as the donor substrate, and as NeuAc transfer is a reversible process, it is difficult to drive the equilibrium in favour of the desired sialoside. For this reason the sialoside donor substrate is regenerated *in situ* by an  $\alpha$ -(2 $\rightarrow$ 3)-sialyltransferase enzyme, thus enhancing the production of the desired product. The specificity of the sialyltransferase ensures that only the galactose byproduct, formed from the sialyl donor, is re-sialylated as the Gal-OR<sub>2</sub> acceptor substrate is a poor substrate. Due to the broad specificity of the *trans*-sialidase, many  $\alpha$ -NeuAc-(2 $\rightarrow$ 3)-Gal-OR sequences can be synthesised by

substituting different galactoside acceptors. The multienzyme system shifts the equilibrium of the *trans*-sialidase towards product formation by the sialyltransferase cycle.

### Materials.

Dimethylethylsilyl $\beta$ -D-galactopyranoside (2.8 mg, 10.5 $\mu$ mol)	assume toxic
Lacto- <i>N</i> -tetraose (0.7 mg, 1.0 $\mu$ mol)	no reported risk
<i>N</i> -Acetylneuraminic acid (sialic acid/NeuAc) (3.2 mg, 10.5 $\mu$ mol)	no reported risk
Cytidine 5'-monophosphate (CMP) (0.3 mg, 0.9 $\mu$ mol)	assume toxic
Adenosine 5'-triphosphate disodium salt (ATP) (0.5 mg, 0.9 $\mu$ mol)	irritant
Phosphoenolpyruvate trisodium salt (10.7 mg, 46 $\mu$ mol)	assume toxic
Magnesium chloride (MgCl <sub>2</sub> ) (1 mg, 10.5 $\mu$ mol)	irritant
Manganese chloride hydrate (MnCl <sub>2</sub> ) (0.6 mg, 3 $\mu$ mol)	harmful, possible risk of irreversible effects
Potassium chloride (KCl) (0.8 mg, 10.5 $\mu$ mol)	assume toxic
Bovine serum albumin (BSA) (5%, v/v, 5 $\mu$ l)	no reported risk
Mercaptoethanol (0.03 $\mu$ l)	irritant, harmful, toxic
Myokinase (15 U)	no reported risk
Pyruvate kinase (25 U)	no reported risk
Inorganic pyrophosphatase (1.6 U)	no reported risk
$\beta$ -(1 $\rightarrow$ 3)(1 $\rightarrow$ 4)-GlcAc $\alpha$ -(2 $\rightarrow$ 3)-sialyltransferase (16 mU)	no reported risk
CMP-NeuAc synthetase (80 mU)	no reported risk
<i>trans</i> -Sialidase from <i>T. cruzi</i> (7 mU) (1 U will sialylate 1 $\mu$ mol of lactose per min at room temperature, pH 7 (1 mM lactose and 1 mM sialyl- $\alpha$ -(2 $\rightarrow$ 3)-lactose)	no reported risk
HEPES buffer (1 ml, 200 mM, pH 7.4)	assume toxic
Methanol for chromatography	flammable, toxic
0.1 M NH <sub>4</sub> HCO <sub>3</sub> for chromatography	harmful if swallowed

### Equipment.

- Round bottomed flask (10 ml) with rubber septum
- Gilson pipettes (100 and 10  $\mu$ l) with tips



Sep-Pack C<sub>18</sub> cartridge (Waters)

Gel filtration (Bio-Gel P2) column chromatography equipment

*Special precautions.* There are no reported risks associated with the enzyme involved; however, many enzymes are immunogenic, so avoid skin contact and inhalation of enzyme powders. Eye protection and gloves should be worn.

*Procedure.* Incubate a solution of dimethylethylsilyl  $\beta$ -D-galactopyranoside (2.8 mg, 10.5  $\mu$ mol), lacto-*N*-tetraose (0.7 mg, 1.0  $\mu$ mol), sialic acid (3.2 mg, 10.5  $\mu$ mol), CMP (0.3 mg, 0.9  $\mu$ mol), ATP (0.5 mg, 0.9  $\mu$ mol), phosphoenolpyruvate trisodium salt (10.7 mg, 46  $\mu$ mol), MgCl<sub>2</sub> (1 mg, 10.5  $\mu$ mol), MgCl<sub>2</sub> (0.6 mg, 3  $\mu$ mol), KCl (0.8 mg, 10.5  $\mu$ mol), BSA (5%, v/v, 5  $\mu$ l), mercaptoethanol (3  $\mu$ l of 1%, v/v solution), myokinase (15 U), pyruvate kinase (25 U), inorganic pyrophosphatase (1.6 U),  $\beta$ -(1  $\rightarrow$  3)(1  $\rightarrow$  4)-GlcAc  $\alpha$ -(2  $\rightarrow$  3)-sialyltransferase (16 mU), CMP-NeuAc synthetase (80 mU), and *trans*-sialidase from *T. cruzi* (7 mU), in 1 ml of HEPES buffer (200 mM, pH 7.4) for four days at room temperature. After this duration, the mixture should be passed through Sep-Pack C<sub>18</sub> cartridge (Waters), washed with 0.1 M NH<sub>4</sub>HCO<sub>3</sub> and eluted with 50% methanol. Pool the fractions containing the product and purify by gel-filtration using a Bio-Gel P2 column and 0.1 M NH<sub>4</sub>HCO<sub>3</sub>. This will yield 1-dimethylethylsilyl-3-*O*-(2- $\alpha$ -*N*-acetylneuraminy)- $\beta$ -D-galactopyranoside (3.9 mg, 65%). <sup>1</sup>H NMR (D<sub>2</sub>O, selected data) 4.44 (d, *J* = 7 Hz, H-1, Gal), 4.04 (dd, *J* = 10, 3 Hz, H-3, Gal), 3.91 (d, *J* = 3 Hz, H-4, Gal), 3.48 (dd, *J* = 10, 7 Hz, H-2, Gal), 2.72 (dd, *J* = 12, 4 Hz, H-3eq, NeuAc), 2.00 (s, NAc), 1.77 (t, *J* = 12 Hz, H-3ax, NeuAc); <sup>13</sup>C NMR (D<sub>2</sub>O) 175.4, 174.3, 102.3 (C-1, Gal), 100.3 (C-2, NeuAc), 76.4 (C-3, Gal), 75.3, 73.3, 72.2, 69.5, 68.8, 68.7, 68.5, 67.9, 63.0, 91.3, 52.1 (C-5, NeuAc), 43.1, 22.5 (COCH<sub>3</sub>), 18.0 (CH<sub>2</sub>SiMe<sub>3</sub>), -2.1 (SiMe<sub>3</sub>).

### 12.2.13 The use of glycosynthases

A new alternative to the reverse hydrolysis and transglycosylation methods of glycosidase-catalysed oligosaccharide synthesis is the use of nucleophile-less glycosidase mutants, glycosynthases [33]. The formation of a glycosynthase from a  $\beta$ -glucosidase/galactosidase from *Agrobacterium* sp. has been described [34, 35]. Replacement of the glutamate active site nucleophile with alanine or serine by site directed mutagenesis resulted in a correctly folded enzyme that was incapable of forming the glycosyl-enzyme intermediate and was therefore catalytically inactive. However, these mutants retain the ability to catalyse the transfer of a sugar moiety from a glycosyl donor (of the opposite anomeric stereochemistry to that of the normal substrate) to an acceptor bound in the aglycon pocket. Figure 12.12 shows the proposed mechanism of glycosynthases.

This catalysis is probably a combination of transition state stabilisation by the intact active site, as well as general base catalysis by the other active site glutamate, enhancing the nucleophilicity of the acceptor. It is postulated [35] that the mutant serine residue stabilises the glycosylation transition state by neutralising

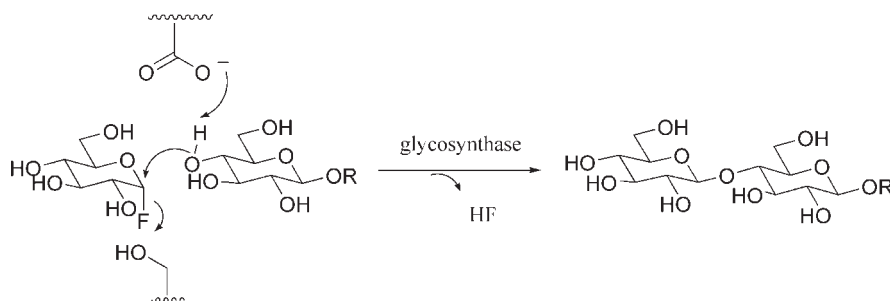


Figure 12.12

the developing negative charge on the departing fluoride by a OH–F hydrogen bond. The utility of glycosynthases offers the dual advantages of using inexpensive glycosyl donors as well as preventing product hydrolysis, consequently increasing yield. A disadvantage of glycosynthases is that the reaction rates are low, requiring substantial amounts of enzyme or long reaction times. Also, unlike other glycosynthetic enzymes mentioned in this chapter, glycosynthases are not yet commercially available and their production using standard molecular biology techniques may be time consuming [36]. However, the serine mutant glycosynthase derived from *Agrobacterium* sp.  $\beta$ -glucosidase has now been recloned into *E. coli* with a His<sub>6</sub>-tag, allowing gram scale production and one step chromatographic purification by metal chelation chromatography [35], suggesting that commercially available sources of various glycosynthases may be available in the not-too-distant future.

### Method 10

Synthesis of 4-methylumbelliferyl 4-*O*-(3-*O*-( $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranoside using a glycosynthase derived from 1,3-1,4- $\beta$ -glucanase from *Bacillus licheniformis* [26] (Figure 12.13).

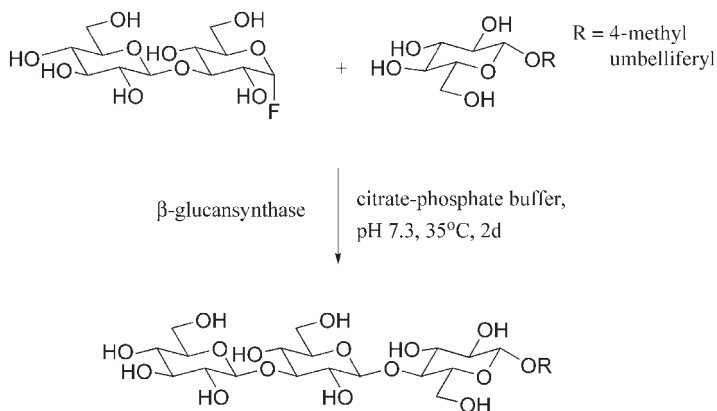


Figure 12.13

*Notes and discussion.* The glycosynthetic potential of *endo*-glycosidases has been largely neglected in the literature. However, unlike *exo*-glycosidases they are not limited to the use of monosaccharide donors and are therefore capable of processing a broader range of substrates. This synthesis uses a glucansynthase, a mutant  $\beta$ -(1  $\rightarrow$  3)(1  $\rightarrow$  4)-glucanase from *B. licheniformis*. Site-directed mutagenesis was used to replace the active site nucleophile, Glu134, with a non-nucleophilic alanine residue, which still allows catalysis of glycosidic bond formation but renders the enzyme incapable of product hydrolysis. This significantly increases transglycosylation yields. This method illustrates the conditions used for a reaction involving a glyco/glucansynthase and demonstrates the value of *endo*-glycosidases in the formation of glycosidic bonds.

#### *Materials.*

4-Methylumbelliferyl $\beta$ -D-glucopyranoside (50 mg, 0.148 mmol)	assume toxic
$\alpha$ -Laminaribiosyl fluoride (10 mg, 0.029 mmol)	assume toxic
E134A $\beta$ -Glucanase (0.5 mg, 20 pmol)	no reported risk
Citrate/phosphate/CaCl <sub>2</sub> buffer (6.1 ml, 6.1 mM/80 mM/0.5 mM, pH 7.3)	assume toxic
Methanol for chromatography	flammable, toxic

#### *Equipment.*

Round bottomed flask (25 ml) with rubber septum  
Syringe (10 ml) and needle  
Water bath  
HPLC column (Lichroprep RP-18 Lobar-A, Merck)  
Rotary evaporator  
Freeze dryer

*Special precautions.* Care must be taken when making the buffer due to salts involved being irritant to the skin and eyes. Avoid inhalation and skin contact with the enzyme. Gloves and eye protection must be worn.

*Procedure.* Suspend 4-methylumbelliferyl  $\beta$ -D-glucopyranoside (50 mg, 0.148 mmol),  $\alpha$ -laminaribiosyl fluoride (10 mg, 0.029 mmol) and E134A  $\beta$ -glucanase (0.5 mg, 20 pmol) in 6.1 mM citrate/80 mM phosphate buffer (6.1 ml, pH 7.3) containing CaCl<sub>2</sub> (0.5 mM) and incubate at 35 °C for two days. After this time, centrifuge the reaction mixture and load the crude extract directly onto a Lichroprep RP-18 (Lobar-A, Merck) HPLC column. Elution with water (250 ml) followed by a gradient elution H<sub>2</sub>O  $\rightarrow$  20% MeOH/H<sub>2</sub>O yields unreacted acceptor, 4-methylumbelliferyl  $\beta$ -D-glucopyranoside (41 mg, 82%) first, followed by the desired disaccharide product. Concentration under reduced pressure and freeze-drying gives

4-methylumbelliferyl 4-*O*-(3-*O*-( $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranoside as a white amorphous solid (17 mg, 88% based on the recovered starting material).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , selected data) 7.70 (d,  $J = 8.8$  Hz, 1H), 7.11 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.05 (d,  $J = 2.1$  Hz, 1H), 6.24 (q,  $J = 0.9$  Hz, 1H), 5.24 (d,  $J = 7.5$  Hz, 1H), 4.77 (d,  $J = 7.8$  Hz, 1H), 4.60 (d,  $J = 7.8$  Hz, 1H), 2.43 (d,  $J = 0.9$  Hz, 3H). MALDI-TOF  $m/z$  685  $[\text{M} + \text{Na}]^+$ .

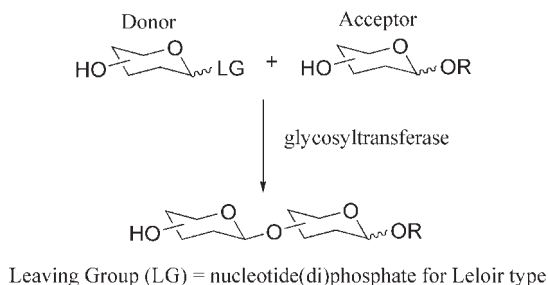
### 12.3 GLYCOSYLTRANSFERASES

Glycosyltransferases (Gly-Ts) essentially construct glycosidic bonds using the same basic approach that has been developed by chemists. They bring together a glycosyl acceptor hydroxyl group to react with a glycosyl donor bearing a good leaving group (Figure 12.14).

For glycosyltransferases the glycosyl donor is usually a glycosyl nucleotidediphosphate (for sialic acid, cytidine monophosphate is used) [37]. The complexity of the nucleotide(di)phosphate leaving group portion gives glycosyltransferases another level of selectivity and particular enzymes not only bind the sugar portion of the glycosyl donor tightly but also the nucleotide portion, in some cases more so. As a result, glycosyltransferases only use certain corresponding pairs of sugar and nucleotide. For example, galactosyltransferases utilize uridine-5'-diphosphogalactose (UDP-Gal) and not GDP or CDP, whereas sialyltransferases use sialic acid donors that contain cytidinemonophosphate (CMP) (Figure 12.15).

This type of glycosyltransferase that uses glycosyl donors with nucleotide mono- or di-phosphate leaving groups at the anomeric centre have been designated *Leloir* type in honour of the Nobel laureate who discovered their mode of action. *Non-Leloir* type use other leaving groups, typically phosphate groups, but their use in synthesis has been limited and therefore they will not be considered here in any detail.

Leloir-type glycosyltransferases are typically membrane bound. In this regard, the use of immobilized enzyme systems [38] may advantageously create enhanced stability; one ready method is the use of affinity supports [39, 40]. In addition, the membrane bound nature of Gly-Ts may require the removal of an enzyme's membrane-binding domain to ensure solubility.



**Figure 12.14**

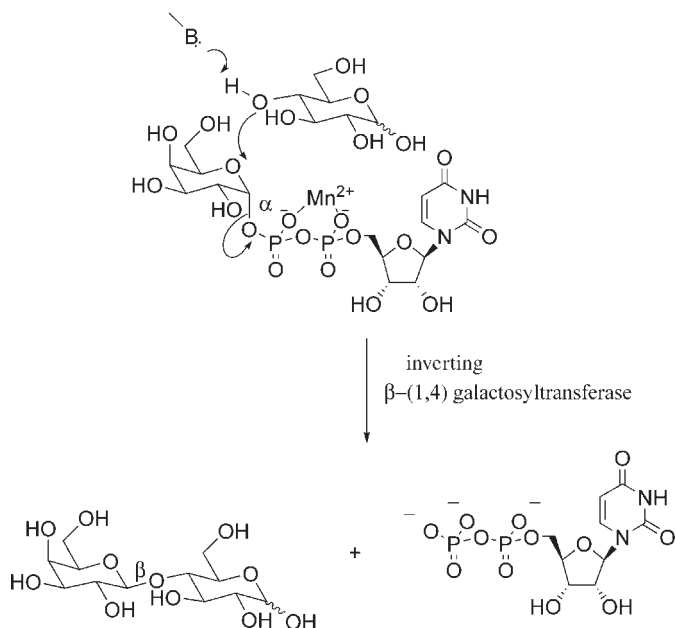


Figure 12.15

The very high specificity of these enzymes for both the substrates that they utilize and the bond-type that they construct (a so-called ‘one enzyme-one linkage’ hypothesis) is such that one-pot systems are readily accessible. One classic example is the synthesis of the important tetrasaccharide sialyl Lewis-x (sLe<sup>x</sup>) [41]: the equivalent using standard chemical techniques required 31 steps! In reality, only a small fraction of the glycosyltransferases in nature has been isolated. Isolation of new glycosyltransferases is sometimes difficult as they may be unstable and are found in only small quantities. Their usual intolerance of substrates that are not their preferred ones also leaves them with potentially limited chemical generality. This limits the use of glycosyltransferases to the formation of the small range of glycosidic bond types that are catalysed by readily available enzymes. However, in this regard it should be noted that some very good examples of probing the limitations of this specificity have been reported in the particular case of  $\beta$ -(1,4)-galactosyltransferase by Palcic and co-workers [42]. Many thousands of putative glycosyltransferase sequences have been identified through various genome sequencing programmes, of which only a very small fraction have been isolated, expressed and functionally characterised. Of these, only a small fraction again have been catalysts for useful synthesis ( $\sim 30$  have now been cloned)  $\alpha$ -galactosyltransferases [27, 43–49],  $\beta$ -galactosyltransferases [47, 50–61],  $\alpha$ -sialyltransferases [50, 57, 59,

62–67],  $\alpha$ -fucosyltransferases [41, 68–72],  $\beta$ -*N*-acetylglucosaminyltransferases [73],  $\beta$ -glucuronyltransferases [74],  $\alpha$ -mannosyltransferases [75, 76] and  $\beta$ -mannosyltransferases [40, 77] with their corresponding donors UDP-Glc, UDP-GlcNAc, UDP-GlcA, UDP-Gal, UDP-GalNAc, UDP-Xyl, GDP-Man, GDP-Fuc, and CMP-NeuAc, respectively.

At the moment there are still only a few commercially available glycosyltransferases and this section will therefore be restricted to those for which a relatively large body of literature and experience exists:  $\beta$ -(1,4)-galactosyltransferases and  $\alpha$ -(2,3)-sialyltransferases. Furthermore, the nucleotide diphosphate glycosyl donors are hard to synthesize, although, as we shall see below other enzymes can be used to overcome this problem and some pragmatic synthetic solutions have also been published [78, 79].

Another potential complicating problem that may be present for all biocatalysis but is often particularly the case for Gly-Ts is that of product inhibition. In the case of Gly-Ts the moderate to occasionally potent  $K_i$  values observed for the nucleotide phosphate leaving groups can therefore limit syntheses, although the use of membrane technology may provide a solution to this in the longer term. In addition, phosphatases can be used to degrade such potential inhibitors. In the following examples, with the robust Gly-Ts described, glass apparatus has been employed, although limited reports have indicated that glass may reduce activity and in these cases plastic containers may be used as a substitute.

A number of very useful reviews covering the use of Gly-Ts have appeared [80–83].

### 12.3.1 Donor synthesis

The major source of nucleotide phosphate ester donors to date has been biocatalysis. Typically, biosynthesis occurs from the corresponding parent carbohydrate through the sequential action of a kinase and then a nucleotide triphosphate pyrophosphorylase. For CMPNeuAc direct transformation occurs through the action of the nucleotide triphosphate pyrophosphorylase (a CMP-NeuAc synthetase). Such systems are sufficiently simple and robust to lend themselves to *in situ* generation of the donor in the presence of Gly-T and this approach, as we shall see below, has greatly enhanced the utility of Gly-Ts in synthesis. *In situ* recycling systems may be further enhanced: the feedback inhibition created by the release of the nucleotide(di)phosphate leaving group may be reduced by the use of phosphatases to break down these side products. For some donors, e.g. GDP-Fuc, biosynthesis occurs through the modification of the sugar portion of another donor. It should be noted that although the cost of commercially available donors and NTPs can in some cases be high, at the time of writing a rapid drop in price is occurring.

Thus, even using such *in situ* technology, most synthetically useful Gly-T catalysed reactions require the use of NTPs as starting materials. Enzymatic

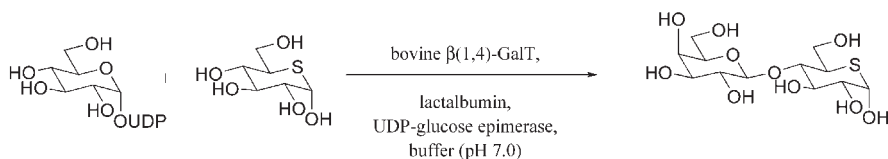
approaches also tend to dominate their synthesis but are beyond the scope of this chapter [84]. As a nice trick, crude mixtures of various NTPs can be selectively converted to a single glycosyl donor through the use of the appropriate pyrophosphorylase, which will specifically process the correct sugar. One limiting factor is that a different pyrophosphorylase is required for each sugar type, although the recent creation of greatly broadened enzymes may provide some hope in this regard [85].

### 12.3.2 Gly-T catalysed glycosylation

Of the glycosyltransferases that are used in synthesis,  $\beta$ -(1,4)-galactosyltransferase, and in particular the bovine milk  $\beta$ -(1,4)-galactosyltransferase ( $\beta$ -(1,4)-GalT, E.C. 2.4.1.22/90) is by far the most widely employed. It was the first to be used in a truly synthetic reaction [86] and the first to be used on a significant (gram) scale [87].  $\beta$ -(1,4)-GalT's acceptor specificity is regulated by the addition of the protein lactalbumin. In the presence of 10% lactalbumin, it forms the lactose synthase complex, which is capable of galactosylating OH-4 of both Glc (E.C. 2.4.1.22 activity) and GlcNAc (E.C. 2.4.1.90 activity), but without lactalbumin the activity towards Glc is lost. Commercial preparations of bovine  $\beta$ -(1,4)-GalT are sold as mixtures with lactalbumin and are available in large quantities at a much lower price than other Gly-Ts (2002 Sigma price £134-70 for 5 units). The substrate specificity of  $\beta$ -(1,4)-GalT has been extensively probed and various experiments have demonstrated a wide tolerance in the donor unit (in Gal OH-6, 4, 3 and 2 may all be deoxygenated, and GalNH<sub>2</sub>, GalNHAc, Glc, GlcNH<sub>2</sub> may be used) and the acceptor unit [42]. Solid-phase galactosylations have also been demonstrated [88, 89].  $\beta$ -(1,4)Gal-T has also been expressed in *E. coli* [90] and yeast [91].

#### Method 11

$\beta$ -(1,4)-Galactosyltransferase-catalysed galactosylation of 5-thioglucose with *in situ* epimerisation of UDP-Glc [51] (Figure 12.16).



**Figure 12.16**

*Notes and discussion.* In this synthetic procedure the addition of UDP-galactose-4-epimerase allows the use of the cheaper donor UDP-Glc rather than UDP-Gal. The moderate yield and relatively long reaction time are a consequence of feedback inhibition from the UDP that is released during transfer which results in incomplete reactions rather than by-product formation; the regeneration/recycling systems

(Methods below) overcome this problem by converting the UDP to UTP but are more complex and require additional components. The procedure is one used in the author's laboratory based on that of Wong and co-workers [51] but incorporates aspects of the procedure of Thiem and Wiemann [56]. Tris instead of cacodylate buffer may also be used. Note that, if available, UDP-Gal could be used directly in this system with the omission of the epimerase.

*Materials.*

5-Thiogluucose (100 mg, 0.5 mmol)	assume toxic
Gal-T (bovine milk, 5 units)	no reported risk
UDP-Glc (350 mg, 0.5 mmol)	assume toxic
Lactalbumin (0.1 mg ml <sup>-1</sup> )	no reported risk
UDP-glucose-4-galactose epimerase (yeast, 10 U)	no reported risk
Sodium cacodylate/MnCl <sub>2</sub> buffer (10 ml, 50 mM/5 mM, pH 7.0)	assume toxic

*Equipment.*

Round bottomed flask (50 ml) with rubber septum  
Bubbler with inert gas supply  
Water bath  
Shaker  
Ion exchange column (Dowex 1, Cl<sup>-</sup> or HCOO<sup>-</sup>)  
Gel filtration column (BioGel P2)  
Rotary evaporator  
Freeze dryer

*Special precautions.* Care must be taken when making the buffer due to the salts involved being irritant to the skin and eyes. Avoid inhalation and skin contact with proteins. Gloves and eye protection must be worn.

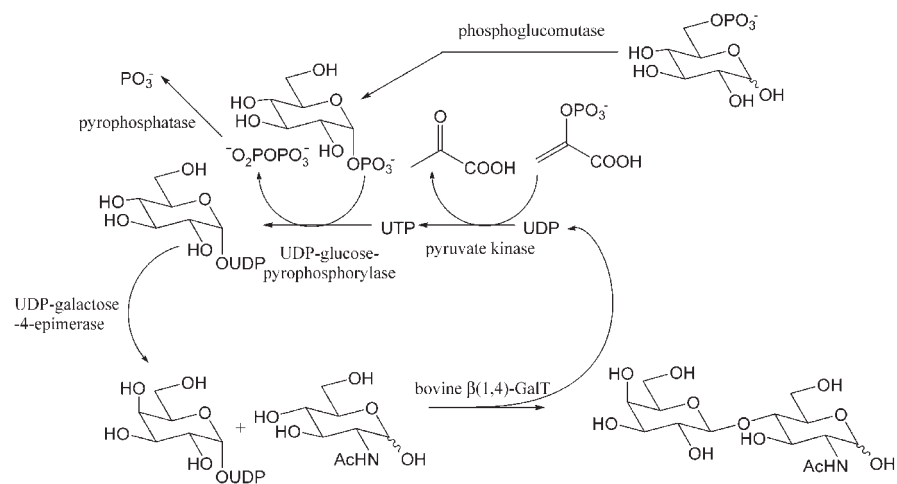
*Procedure.* Dissolve 5-thiogluucose (100 mg, 0.5 mmol), UDP-Glc (350 mg, 0.5 mmol) and lactalbumin (0.1 mg ml<sup>-1</sup>) in sodium cacodylate/MnCl<sub>2</sub> buffer (10 ml, 50 mM/5 mM, pH 7.0) and degass the resulting solution using argon. Add the enzymes GalT (5 U) and UDP-glucose-4-galactose epimerase (10 U) and shake at 37 °C for two days. The reaction course can be conveniently monitored by TLC. (CHCl<sub>3</sub>/MeOH/AcOH/H<sub>2</sub>O or PrOH/AcOH/H<sub>2</sub>O): two days typically corresponds to approximately 50% conversion. After this time, the reaction mixture may be loaded onto a Dowex 1 column (Cl<sup>-</sup> or HCOO<sup>-</sup>, ~50 ml column volume) and eluted with water (300–400 ml). Reduce the reaction volume to ~5–10 ml and then load onto a BioGel P2 gel filtration column (4 × 100 cm) and elute with water. Freeze-drying gives 4-*O*-(β-D-galactopyranosyl)-5-thio-D-glucopyranose as a white amorphous solid (96 mg, 53%). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) 4.95 (d, 1H, *J* = 3 Hz,



H-1 $\alpha$ ), 4.51 (d, 1H,  $J = 8$  Hz, H-1'), 4.05 (dd, 1H,  $J = 5, 12$  Hz, H-6a), 3.83–3.9 (m, 3H, H-4, H-6b, H-4'), 3.65–3.75 (m, 4H, H-3, H-5', H-6a', H-6b'), 3.62 (dd, 1H,  $J = 10, 3.5$  Hz, H-3'), 3.53 (dd, 1H,  $J = 3, 10$ , H-2'), 3.32 (ddd, 1H,  $J = 2.5, 5, 10.5$ , H-5);  $^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ ) 103.2 (C-1'), 82.3 (C-4), 75.8 (C-5'), 75.7 (C-2), 73.3 (C-1), 73.0 (C-3), 72.7 (C-3'), 71.7 (C-2'), 69.0 (C-4'), 61.5 (C-6'), 59.7 (C-6), 42.5 (C-5); HRMS  $m/z$  ( $\text{M} + \text{Cs}^+$ ) calc. 490.9988, found 491.0022.

### Method 12

Supported  $\beta$ -(1,4)-galactosyltransferase-catalysed galactosylation of *N*-acetylglucosamine using *in situ* recyclisation of UDP-Glc by UDP-glucose-pyrophosphorylase [56] (Figure 12.17).



**Figure 12.17**

**Notes and discussion.** This procedure is based on the protocol of Thiem and Wiemann [56], with some corrections and adaptations, although the use of such a six-enzyme pyrophosphorylase-recycled system was first proposed by Whitesides and Wong [87]. It recycles the UDP side product of Gal-T-catalysed synthesis using pyruvate kinase to create UTP; this helps to reduce feedback inhibition by UDP. The UTP produced is then reacted with glucose-1-phosphate catalysed by the action of UDP-glucose-pyrophosphorylase to regenerate Glc-UDP before epimerisation by the epimerase to Gal-UDP. Addition of pyrophosphatase renders this transfer essentially irreversible by hydrolysing the pyrophosphate formed. Glucose-1-phosphate may be synthesised from the more readily available glucose-6-phosphate under the catalysis of phosphoglucomutase. Immobilization of the enzymes involved, here using CH-Sepharose 4B (Pharmacia), allows their reuse, increases the economic efficiency and as a result the reaction may be stopped simply through filtration. However, it should be noted that immobilization also prevents the formation of the lactose

synthase complex and therefore limits acceptor specificity to GlcNAc systems. This protocol may be readily scaled to the multigram scale.

Finally, it should be noted that an alternative recycling system that utilises galactose kinase in conjunction with galactose-1-phosphate uridylyltransferase as an effective replacement for the epimerase mediated conversion of Glc-UDP to Gal-UDP has also been described [92]. In this system, galactose kinase (5 U) and galactose-1-phosphate uridylyltransferase (5 U) replace the epimerase system; pyruvate kinase levels are doubled (to 100 U) as this enzyme plays the additional role of regenerating ATP from ADP. This elegant system advantageously allows the use of just D-galactose as a starting material.

#### *Materials.*

GlcNAc (0.4 mmol)	no reported risk
Glucose-6-phosphate (0.4 mmol)	assume toxic
KCl (55 mg, 0.74 mmol)	assume toxic
Phosphoenol pyruvate (PEP, 87 mg, 0.42 mmol)	assume toxic
UTP (56 mg, 0.01 mmol)	assume toxic
BSA (10 mg)	no reported risk
Tris/MnCl <sub>2</sub> /MgCl <sub>2</sub> buffer (20 ml, 100 mM/0.1 mM/0.2 mM, pH 7.5)	assume toxic
Phosphoglucomutase (rabbit muscle, 50 U)*	no reported risk
UDP-glucose-pyrophosphorylase (bovine liver, 10 U)*	no reported risk
Pyrophosphatase (yeast, 30 U)*	no reported risk
UDP-glucose-4-galactose epimerase (yeast, 5 U)*	no reported risk
Gal-T (bovine milk, 5 U)*	no reported risk
Pyruvate kinase (rabbit muscle, 50 U)*	no reported risk
Activated CH-Sepharose 4B	assume toxic, irritant

Asterisk denotes an immobilised enzyme.

#### *Equipment.*

Round bottomed flask (50 ml) with rubber septum  
 Bubbler with inert gas supply  
 Water bath  
 Shaker  
 Sinter funnel or similar medium porosity filtration apparatus  
 Ion exchange column (Dowex 1, Cl<sup>-</sup> or HCOO<sup>-</sup>)  
 Gel filtration column (BioGel P2)

Rotary evaporator

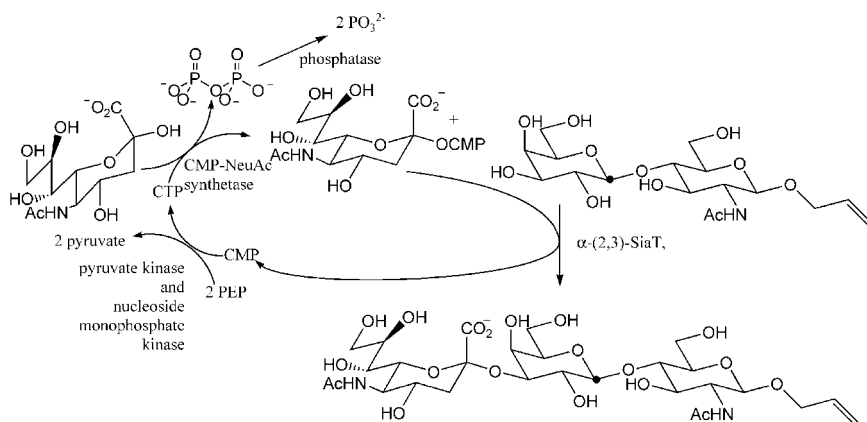
Freeze dryer

*Special precautions.* Care must be taken when making the buffer due to salts involved being irritant to the skin and eyes. Avoid inhalation and skin contact with proteins. Gloves and eye protection must be worn. Note that the CH-Sepharose 4B is prepared by activation with CNBr, which is highly toxic, and in our hands loading yields of enzyme onto this support decrease significantly 15 min after swelling due to hydrolysis.

*Procedure.* Immobilise all enzymes using activated CH-Sepharose 4B according to the manufacturer's instructions (Pharmacia). Approximately, 40 mg of protein can be immobilised on about 1 ml of swollen CH-Sepharose 4B gel. 250 mg of CH-Sepharose 4B powder yields about 1 ml of swollen gel. Briefly, the treatment process involves swelling and washing the gel powder with ice cold, dil. HCl (aqueous, 1 M) immediately prior to coupling. Dissolve GlcNAc (0.4 mmol), glucose-6-phosphate (0.4 mmol), KCl (55 mg, 0.74 mmol), PEP (87 mg, 0.42 mmol), UTP (56 mg, 0.01 mmol), BSA (10 mg) in Tris buffer (20 ml, 100 mM, pH 7.5 containing 0.1 mM  $\text{MnCl}_2$ , 0.2 mM  $\text{MgCl}_2$ ) and degass using the inert gas supply and bubbler. Add the immobilized enzymes: phosphoglucosyltransferase (rabbit muscle, 50 U), UDP-glucose-pyrophosphorylase (bovine liver, 10 U), pyrophosphatase (yeast, 30 U), UDP-glucose-4-galactose epimerase (yeast, 5 U), Gal-T (bovine milk, 5 U), pyruvate kinase (rabbit muscle, 50 U) and shake at 37 °C for seven days. The reaction course can be conveniently monitored by TLC ( $\text{CHCl}_3/\text{MeOH}/\text{AcOH}/\text{H}_2\text{O}$  or  $\text{PrOH}/\text{AcOH}/\text{H}_2\text{O}$ ): 3–5 days typically corresponds to approximately 50% conversion. After this time, the reaction mixture can be filtered using a sinter funnel to remove the supported enzymes and to therefore stop the reaction. After washing with water and then reaction buffer (~200 ml of each) the immobilized enzymes may be used in the next reaction (typically up to five re-uses can be expected before fresh enzyme is needed although prolonged storage between runs may reduce activity). Load the filtrate onto a Dowex 1 ( $\text{Cl}^-$  or  $\text{HCOO}^-$ , ~50 ml column volume) column and elute with water (300–400 ml). Reduce the reaction volume to ~5–10 ml and then load onto a BioGel P2 gel filtration column (4 cm  $\times$  100 cm) and elute with water. Freeze-drying gives 4-O-( $\beta$ -D-galactopyranosyl)-2-acetamido-2-deoxy-N-acetylglucosamine as a white amorphous solid (0.31 mmol, 78%).  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ , selected data) 5.19 (d, 1H,  $J = 10$  Hz, H-1 $\alpha$ ), 4.71 (d, 1H,  $J = 7.5$  Hz, H-1 $\beta$ ), 4.46 (d, 1H,  $J = 7.5$  Hz, H-1'), 3.95 (dd, 1H,  $J = 3.8, 10.1$  Hz, H-6a), 3.53 (ddd, 1H,  $J = 3.8, 7.7, 10.1$  Hz, H-5), 3.65 (ddd, 1H,  $J = 1.7, 3.1, 10.2$  Hz, H-5'), 2.03 (s, 3H, NAc);  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ) 176.0 ( $\text{CH}_3\text{CO}$ ), 104.1 (C-1'), 96.2 (C-1 $\beta$ ), 91.6 (C-1 $\alpha$ ), 80.1 (C-4 $\alpha$ ), 79.7 (C-4 $\beta$ ), 76.6 (C-5'), 76.1 (C-5 $\beta$ ), 74.9 (C-3 $\beta$ ), 73.9 (C-3'), 72.3 (C-2'), 71.5 (C-5 $\alpha$ ), 70.6 (C-3 $\alpha$ ), 69.9 (C-4'), 62.3 (C-6'), 61.3 (C-6 $\alpha$ , C-6 $\beta$ ), 57.6 (C-2 $\beta$ ), 55.1 (C-2 $\alpha$ ), 23.3 ( $\text{CH}_3\text{CO}-\alpha$ ), 23.5 ( $\text{CH}_3\text{CO}-\beta$ ).

*Method 13*

Sialylation of allyl 4-*O*-(3-*O*-(2- $\alpha$ -N-acetylneuroaminyl)- $\beta$ -D- $^{13}$ C-1-galactopyranosyl)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside using an  $\alpha$ -2,3-sialyltransferase with *in situ* regeneration of CMP-NeuAc [41] (Figure 12.18) .



**Notes and discussion.** This procedure is taken from Wong and co-workers' landmark publication [41] on the sequential use of GalT, SiaT and FucT to synthesise sialyl Lewis-x from the corresponding parent carbohydrates and allyl GlcNAc as the starting acceptor. This method reports the intervening sialylation and utilises a recombinant source of the catalytic domain of rat liver  $\alpha$ -2,3-sialyltransferase [93] expressed in baculovirus. The parallel use of an  $\alpha$ -2,6-sialyltransferase has also been described and the enzymes are essentially interchangeable in the procedure below [94, 95]. The commercial availability is variable and at the time of writing both were available on a unit scale. The recycling system uses a NeuAc-synthetase to make the donor from sialic acid in one step; the CMP is converted to CTP through the sequential action of two kinases. Again, the availability of these enzymes may vary; in the absence of a recycling system stoichiometric quantities of CMP-NeuAc may be used in conjunction with phosphatases to circumvent feedback inhibition that may result in lower yields [96]. It should be noted that in some cases higher yields may be obtained by longer reaction times (e.g. nine days) and the addition of repeated doses of the enzymes.

*Materials.*

Allyl $^{13}\text{C}$ -1-Gal-( $\beta$ -1,4)-GlcNAc (310 mg, 0.5 mmol)	assume toxic
NeuAc (160 mg, 0.52 mmol)	no reported risk
Phosphoenol pyruvate, sodium salt (PEP, 120 mg, 0.51 mmol)	assume toxic
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (4.9 mg, 0.025 mmol)	assume toxic

KCl (7.5 mg, 0.10 mmol)	assume toxic
CMP (16 mg, 0.05 mmol)	assume toxic
ATP (2.7 mg, 0.005 mmol)	assume toxic
$\beta$ -Mercaptoethanol (0.34 $\mu$ l)	toxic
HEPES buffer (3.5 ml, 200 mM, pH 7.5)	assume toxic
NaOH (aqueous, 1 M)	harmful
Nucleoside monophosphate kinase (NMK, 5 U)	no reported risk
Pyruvate kinase (rabbit muscle, 100 U)	no reported risk
Pyrophosphatase (yeast, 10 U)	no reported risk
CMP-NeuAc synthetase (0.4 U)	no reported risk
$\alpha$ -(2,3)-Sia-T (0.1 U)	no reported risk

#### *Equipment.*

Round bottomed flask (25 ml) with rubber septum  
 Bubbler with inert gas supply  
 Shaker  
 Gel filtration column (BioGel P2)  
 Rotary evaporator  
 Freeze dryer

*Special precautions.* Care must be taken when making the buffer due to the salts involved being irritant to the skin and eyes. Avoid inhalation and skin contact with proteins. Gloves and eye protection must be worn.

*Procedure.* Dissolve allyl  $^{13}\text{C}$ -1-Gal-( $\beta$ -1,4)-GlcNAc (310 mg, 0.5 mmol), NeuAc (160 mg, 0.52 mmol), phosphoenol pyruvate, sodium salt (PEP, 120 mg, 0.51 mmol),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (4.9 mg, 0.025 mmol), KCl (7.5 mg, 0.10 mmol), CMP (16 mg, 0.05 mmol), ATP (2.7 mg, 0.005 mmol), and  $\beta$ -mercaptoethanol (0.34  $\mu$ l) in HEPES buffer (3.5 ml, pH 7.5) and adjust back to pH 7.5 with NaOH (aqueous, 1 M). Add all the enzymes and shake under argon at 25 °C for three days. After this time, remove the solvent and purify by flash chromatography ( $\text{EtOAc}:\text{iPrOH}:\text{H}_2\text{O}$ , 2:2:1) and then BioGel P2 chromatography (eluant  $\text{H}_2\text{O}$ ) followed by freeze drying to give the trisaccharide as a white solid (88 mg, 24%).  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ , selected data) 4.58 (d, 1H,  $J$  = 8.3 Hz, H-1 GlcNAc), 4.56 (dd, 1H,  $J$  = 7.8 Hz, H-1 Gal), 4.13 (dd, 1H,  $J$  = 3.1, 9.9 Hz, H-3 Gal), 3.96 (br d, 1H,  $J$  = 3.1 Hz, H-4 Gal), 2.76 (dd, 1H,  $J$  = 4.6, 12.3 Hz, H-3b NeuAc), 2.04 (s, 6H,  $\text{NHAc} \times 2$ ), 1.84 (br t, 1H,  $J$  = 12.0 Hz, H-3a NeuAc); HRMS  $m/z$ : calcd  $\text{C}_{27}\text{H}_{44}\text{N}_2\text{O}_{19}\text{Cs}_2$  ( $\text{M} - \text{H}^+ + 2\text{Cs}^+$ ) 980.0759; found 980.0720.

**Table 12.2**

A broad comparison of the utility of glycosyltransferases and glycosidases

	Glycosyltransferases	Glycosidases
Efficiency in disaccharide synthesis	Usually > 80%	Often $\leq 40\%$ <sup>a</sup>
Specificity	For the glycosyl donor, glycosyl acceptor and the nucleotide leaving group	For the glycosyl donor <sup>b</sup> and sometimes partially for the glycosyl acceptor
Ease of use	Need buffers, correct glycosyl donor, harder to isolate, product inhibition.	Often robust, tolerant of conditions, easier to isolate.
Commercial availability	< 15 <sup>c</sup>	50–100 <sup>c</sup>

<sup>a</sup>Note that yields for the use of glycosynthases can exceed 90% [33].<sup>b</sup>Progress is now being made to loosen this overly stringent specificity [97].<sup>c</sup>In 2002.

## 12.4 OVERALL SUMMARY

When deciding whether to use a glycosyltransferase or a glycosidase enzyme in disaccharide synthesis it should be remembered that each have their advantages and disadvantages, some of which are summarised in Table 12.2. These often complement rather than oppose each other, and these two types of enzymes may even be used together in synthetic routes to take advantage of their particular strengths.

As a general rule, the current state of play for carbohydrate biocatalysis is that certain disaccharidic motifs are constructed by default using enzymes e.g. the  $\alpha$ -(2,3)-sialyl-galactose linkage formed using  $\alpha$ -(2,3)-sialyltransferase is now typically used as a final synthetic elaborative adjunct to many purely chemical routes [96]. For some, such as the  $\beta$ -galactoside linkage, a wider choice of similarly pragmatic chemical and biocatalytic methods (e.g.  $\beta$ -galactosidases,  $\beta$ -(1,4) or  $\beta$ -(1,3) galactosyltransferases, or participatory chemical galactosyl donors) exist. For yet others, such as the infamous  $\beta$ -mannoside linkage, no one general method is yet available. Thus, although the availability of these enzymes makes biocatalysis a more-and-more pragmatic solution to glycosylation—if a biocatalytic method exists it will tend to be quicker, easier and higher yielding—at present, this must be supplemented by a wise bond-type by bond-type selection.

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# Index

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## A

$\alpha$ -2,3-sialyltransferase 421  
 $\alpha$ -fucosidase 393, 394  
 $\alpha$ -mannoside linkage 241  
*A. oryzae* 393  
acetates 23, 25, 69, 70  
acetimidates 220  
acetobromoglycoses 69  
AgOTf 205, 210, 222  
*Agrobacterium* sp. 410  
AIDS 339  
alkyl carbonate 28  
allyl (All) 12  
allyl ethers 12, 13, 17  
almond  $\beta$ -D-glucosidase 396  
1,2-anhydride/glycosyl epoxides 69  
1,2-anhydrosugars 109, 367  
anomeric acetates 69, 74  
anomeric acetimidates 148  
anomeric effect 241  
anomeric glycosyl azides 318  
anomeric glycosylamines 318  
anomeric isothiocyanates 318  
anomeric *n*-pentenyl glycosides 318  
anomeric nitro sugars 344  
anomeric radical 344  
anomeric samarium species 351  
anti-infective agents 2  
antigen Globo-H 207, 222  
appended type donors 286  
armed (activated) donors 197  
armed–disarmed 105, 131, 136, 179, 180  
*Asperigillus oryzae* 402  
avermectin B<sub>1a</sub> 222  
axial hydroxyls 11  
2-azido-2-deoxy-D-galactose 314  
azidophenylselenation 100

## B

1,2- $\beta$ -elimination 340  
 $\beta$ -galactosidase 393, 406  
 $\beta$ -glycosidase 395  
 $\beta$ -mannoside(s) 137, 213, 239  
 $\beta$ -*N*-acetylhexosaminidase 393  
*B. circulans* 393  
*Bacillus circulans* 406  
*Bacillus licheniformis* 411  
BDA 213  
benzoate esters 27  
benzoyl (Bz) 313  
benzyl (Bn) 12, 313  
benzyl ethers 12, 13  
benzylidene 30, 31, 201  
biosynthesis 4  
bovine kidney 393  
bovine milk  $\beta$ -(1,4)-galactosyltransferase 416  
bovine testes 393  
bromides 69  
Bu<sub>2</sub>Sn(OTf)<sub>2</sub> 97  
butanediactal (BDA) 32, 89, 201

## C

cancer mucins 312  
*C,C*-trisaccharide 339  
*C*-disaccharide 339  
*C*-glycosides 337  
*C*-saccharides 339  
CAN 17  
carbohydrate-directed therapies 4  
carbohydrate-processing enzymes 386  
CDA 212, 215  
CDP 413  
cell adhesion 337  
cell differentiation 337  
cell growth 1

cell recognition 337  
 cell-external agent interactions 1  
 cell-surface membranes 1  
 cell-cell recognition 1  
 chitobiose pentasaccharide core 317  
 chlorides 69  
 chloroacetyl 25  
 $\text{Cp}_2\text{HfCl}_2/\text{AgOTf}$  225  
 $\text{Cp}_2\text{HfCl}_2$  205  
 cyclic carbonates 28, 29  
 cyclic orthoesters 271  
 cyclic oxonium 196  
 cyclohexanediactal 32, 201  
 cytidinemonophosphate (CMP) 413

## D

DAST/hypervalent iodoarene difluoride 130  
 3-deoxy-non-2-ulonic acid 277  
 DCC 319  
 DDQ 17, 267  
 dibutylstannylene complexes 254  
 dicobalthexacarbonyl complex 368  
 diethylamino sulfurtrifluoride (DAST) 85  
 differentiation 1  
 2,2-dimethyldioxirane (DMDO) 116  
 diphenylmethyl (benzhydryl) ethers 16  
 disarmed (deactivated) acceptors 197  
 dispiroketal(s) 32, 201  
 1,3-dithian-2-ylmethyl (Dim) 319  
 donor reactivities 211

## E

*E. coli* 393  
 EEDQ 319  
 electronic effects 197, 198, 206  
 electronic reasons 226  
 endoplasmic reticulum 4  
 energy storage 1  
 envelope glycoprotein gp120 211  
 enzymes 386  
 equatorial positions 11  
*Escherichia coli* 400  
 everninomicin 223, 224  
 Everninomicins 239

## F

fertilization 1  
 Fischer glycosylation 166, 168  
 9-fluorenylmethoxycarbonyl (Fmoc) 313  
 fluorides 69  
 furanose 5

## G

GDP 413  
 Globo H glycolipid 199  
 glycals 109  
 glycoprocessing 386  
 glycosidase enzymes 337  
 glycosidases 386, 387  
 glycosyl acceptor 5  
 glycosyl amino acids 311  
 glycosyl bromides 69, 77, 130, 221, 227  
 glycosyl bromides/halides 76  
 glycosyl chlorides 77, 126, 130  
 glycosyl donor reactivity 198  
 glycosyl donor 5  
 glycosyl fluorides 76, 84, 130, 201, 204, 205, 222, 223, 225, 229, 230  
 glycosyl halides 168, 197, 220  
 glycosyl iodides 76  
 glycosyl phosphates 259  
 glycosyl sulfoxides 137  
 glycosyl thioacetates 127  
 glycosylhydrolases 386  
 glycosylphosphatidylinositol (GPI) anchor 213  
 glycosyltransferases 338, 386  
 glycosynthases 410  
 golgi apparatus 4  
 GPI anchor 213, 214, 222  
 gram-negative bacteria 239

## H

hafnocene dichloride ( $\text{Cp}_2\text{HfCl}_2$ )/ $\text{AgClO}_4$  222  
 hafnocene dichloride 204, 231, 235, 236  
 haloglycosylation 114  
 hermaphrodites 196  
 high-mannose type neoglycolipids 223  
 human blood groups 2  
 human breast milk 2  
 human deficiency virus (HIV) 211

## I

IDCP: iodonium dicollidine perchlorate 197  
 IDCP 202, 203, 206, 208, 209  
 IIDQ 319  
 immune responses 1  
 inflammation 337  
 influenza 339  
 insoluble promoters 242  
 insoluble silver salt 242  
 intramolecular aglycon delivery 93, 261  
 intramolecular glycosylation reaction 261  
 isopropenyl glycosides 182

isopropylidene 30, 31, 201  
iterative block synthesis 222

**K**

Koenigs–Knorr 69, 82, 147, 166, 168, 177, 242,  
248, 295, 313

**L**

lactols 355  
lactones 358  
L-asparagine (Asn) 311  
latent/active 172, 179, 184, 185, 188  
lectins 1, 337  
Leloir-type glycosyltransferases 413  
levels of reactivity 198, 225  
levulinate 26  
lipases 24  
L-serine (Ser) 311  
L-threonine (Thr) 311

**M**

mannosyl bromide 243  
masked formyl group 358  
methyl ethers 11  
migration 27  
molecular recognition processes 1  
mucins 311  
mutarotation 150

**N**

N-bromosuccinimide (NBS) 197  
N-iodosuccinimide 199, 214, 222, 229, 232, 233,  
235  
N-linked glycopeptides 311  
N-linked glycoproteins 239  
n-pentenyl glycosides 165, 197  
n-pentenyl groups 148  
n-pentenylloxymethyl-based protecting group 165  
NBS/DAST 222  
neighboring group participation 222, 241  
neuraminic acid 277  
NIS/AgOTf 222  
NIS/TfOH 201, 212, 224, 225  
NIS 215, 236  
Non-Leloir type 411

**O**

O-linked glycopeptides 311  
one-pot 198, 199, 201, 206, 207, 211, 212, 222,  
225, 226, 232  
organostannanes 341

orthoester(s) 23, 96, 271  
orthogonal activation 225  
orthogonal glycosidation 195, 219, 220  
orthogonal 235  
2-oxo bromide 246  
oxone 252  
oxonium ion 196

**P**

*P. multicolor*  $\alpha$ -L-fucosidase 404  
*P. multicolor* 393  
*Penicillium multicolor* 394  
4-pent-1-yl acceptor 224  
pentafluorophenyl (Pfp) ester 314  
4-penten-1-yl glycosides 131, 220, 223  
4-penten-1-yl 221  
pentenyl donor 202  
peracylated furanose derivatives 70  
phenyl selenoglycosides 69, 96, 102, 212  
pivaloate 25  
PMB ethers 267  
polysaccharide-antigen Group B  
*Streptococci* 201  
protecting group chemistry 7, 9  
protecting group 5, 10  
pyranose 5

**R**

reactivity tuning 225, 235  
reductive cleavage 271  
reductive lithiation 340  
ring closing metathesis 368

**S**

*S. solfataricus* 395  
selectins 2  
seleno- 220  
selenoglycoside 95, 198, 201, 209, 213, 223  
sequential glycosidation 222  
sequential glycosylation 211, 222  
sialic acids 277  
sialyl Lewis X hexasaccharide 303  
sialyltransferase 408  
silver carbonate 242  
silver imidazolate 242  
silver perchlorate 231  
silver salicylate 242  
silver tosylate 242  
silver triflate 213  
silver trifluoromethanesulfonate 204, 210, 221,  
229, 235, 236  
silyl acetal tether 263

silyl acetals 261  
silyl ethers 20  
silyl tether 375  
skeletal components 1  
 $\text{SnCl}_2/\text{AgClO}_4$  222  
solid phase synthesis 7, 322  
standard type donors 278  
stannylene acetal 17, 256  
steric effects 206  
sugar aldehydes 365  
*Sulfolobus solfataricus* 395  
sulfoxide glycosylation methodology 251  
sulfoxides 220, 224

**T**  
 $T_N$  antigen 325  
Tebbe methylenation 183  
*tert*-butyldimethylsilyl 20  
*tert*-butyldiphenylsilyl 20  
thioglycoside 121, 128, 201, 206, 213, 220, 221, 223, 224, 227, 229, 230  
Thomsen–Freidenreich ( $T_F$ )-antigen 312  
torsional and electronic effects 196  
torsional effects 199, 225  
*trans*-sialidase 408  
transglycosylation 388  
transmetallation 341  
trichloroacetimidate 148, 224, 232

trifluoromethanesulfonic acid 214, 215, 235  
trimethylsilyl trifluoromethanesulfonate 92, 224, 232, 233  
triphenylmethyl (tritylTr) 16  
*Trypanosoma brucei* 213, 222  
tumour associated carbohydrate antigens 2  
tumour metastasis 337  
tuning of reactivity 195, 219  
two-stage activation 222  
two-stage glycosidation 229  
two-step orthogonal glycosidation 232

**U**

UDP-Glc 418  
2-ulosyl bromides 246  
uridine-5'-diphosphogalactose 413

**V**

vinyl glycosides 148, 181  
viral and bacterial infections 337

**W**

Wilkinson's catalyst 184, 266  
Wittig olefination 361

**Z**

Zemplén deprotection 24, 26, 109